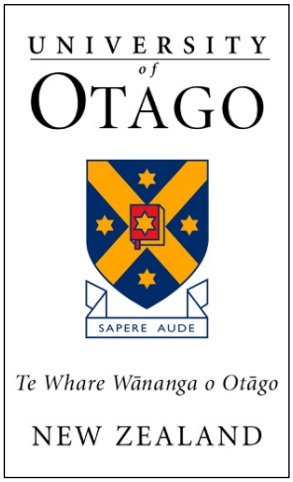
The Role of Calcium-Permeable AMPA Receptors and Arc in Secreted Amyloid Precursor Protein Alpha-Mediated Plasticity

Rhys Warren Livingstone



A thesis submitted for the degree of
Doctor of Philosophy
at the University of Otago,
Dunedin, New Zealand

August 2020

Abstract

The orchestrated regulation of the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)-subtype of glutamate receptors by neuronal activity and neuromodulators is critical to the expression of both long-term potentiation (LTP) and memory. In particular, GluA1-containing, Ca^{2+}-permeable AMPAR (CP-AMPAR) comprise a unique role in these processes due to their transient, activity-regulated expression at the synapse. Importantly, many of the mechanisms which govern these processes are negatively affected in neurodegenerative disorders such as Alzheimer's disease, suggesting that understanding the mode of action of neuromodulatory molecules may reveal much needed novel therapeutic interventions. Secreted amyloid precursor protein-alpha (sAPPα), a metabolite of the parent amyloid precursor protein (APP) has been previously shown to enhance hippocampal LTP and facilitate memory formation. Accordingly, we hypothesised that sAPPα may act via modulation of AMPAR synthesis and cell surface expression.

Using primary hippocampal neurons grown in culture, we found that sAPPα (1 nM) differentially regulates the expression of cell surface GluA1-, GluA2-, and GluA3-containing AMPAR. Interestingly, using fluorescent non-canonical amino acid tagging with proximity ligation assay (FUNCAT-PLA), we found that short-term sAPPα treatments (1 nM, 30 min) rapidly enhanced the cell surface expression of newly synthesised extrasynaptic GluA1-, but not GluA2-containing AMPAR, while long-term treatments of sAPPα (1 nM, 120 min) increased levels of pre-existing GluA1/2-containing heteromers at the cell surface, indicating a dynamic regulation of distinct AMPARs following treatment. Moreover, using electrophysiology in area CA1 of acute hippocampal slices, we provide evidence that the expression of CP-AMPAR is important in the induction of sAPPα-enhanced LTP. Using immunocytochemistry and siRNA knockdown, we provide evidence that internalization of CP-AMPARs may be governed, at least in part by sAPPα-driven expression of the activity-regulated cytoskeletal-associated protein (Arc). Further, we show that Arc expression is not induced by the related APP metabolite sAPPβ, but is dependent on synergistic activation of N-Methyl-D-Aspartate and α7-nicotinic acetylcholine receptors, as well as downstream activation of CaMKII, MAPK, and PKG.

Together, these findings suggest that application of sAPPα to hippocampal neurons engages a cascade of mechanisms which enhance the synthesis and expression of AMPAR and Arc protein, in the regulation of synaptic strength and the expression of hippocampal LTP. These experiments expand upon our current knowledge underlying mechanisms of synaptic plasticity in hippocampal neurons.

Acknowledgements

To all of those who have helped me along the way.

The team at the Hercus Taieri Resource Unit, for your patience and care in the handling of all animals used throughout our experiments.

The people of the Department of Anatomy, Scott115, and the cell culture room, for providing an environment I enjoyed to be a part of.

The Abraham group, especially Anurag and Owen. Thank you for introducing me to the world of electrophysiology, guiding me through, and answering my many questions. To Bruce, for being a bouncing board of ideas and problem solving for all the cell culture woes we experienced.

The Williams lab, past and present. Thank you from the bottom of my heart. Thank you for the all the support you have provided whether that be technical or emotional, it was all appreciated, always. To Megan, for introducing me to science all those years ago, and encouraging me non-stop throughout. To Diane, for understanding when others wouldn't, and for helping when others couldn't. You make the lab feel like home. To Margaret, for your unwavering support and many sAPPα-related conversations. To Aimee, for the shared frustrations that come with cell culture and the appreciation of all things LTP. To Nik and Rebecka, for the support, in all they ways that took. To Courteney, for trusting me to teach you the ways of cell culture, I wish you the best of luck. To Joyeeta, for being a friend, a companion, and an inspiration. You made the tough times easier.
I thank you all for this.

My friends, for taking the time to understand what I do, and supporting me throughout.

My supervisors Jo and Cliff. To Cliff, for your knowledge and expertise throughout my electrophysiology experiments and beyond. To Jo, for inviting me into your lab all those years ago. Your passion, knowledge, and support have been a strong source of motivation throughout. Thank you.

Finally, to my family. Your unwavering support, patience, and understanding have sometimes been all that kept me going. To Nana and Granddad. Although you never quite understood what I was doing, you supported me wholeheartedly. For this, I dedicate this work to you.

Table of Contents

Table of Figures

Table of Tables

Abbreviations

5-HT	5-Hydroxytryptamine
aCSF	Artificial Cerebrospinal Fluid
Act-D	Actinomycin-D
AD	Alzheimer's Disease
ADF	Actin Depolymerizing Factor
AHA	L-Azidohomoalanine
AICD	APP Intracellular Domain
AKAP79/150	A-kinase Anchoring Protein 79
AMPAR	α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid Receptor
Aniso	Anisomycin
APLP1/2	Amyloid Precursor-Like Proteins 1/2
APOE	Apolipoprotein E
APP	Amyloid Precursor Protein
APPL2	APP-like protein 2
APV	D(−)-2-Amino-5-Phosphonopentanoic Acid
Ara-C	Cytosine arabinoside
Arc	Activity-Regulated Cytoskeletal-Associated Protein
ATD	Amino Terminal Domain
Aβ	Amyloid-Beta
BACE1	β-site Amyloid Precursor Protein-Cleaving Enzyme 1
BBB	Blood-Brain Barrier
BCM	Bienenstock, Cooper and Munro
BD	Bipolar Disorder
BDNF	Brain Derived Neurotrophic Factor
bFGF	Basic Fibroblast Growth Factor
Bic	Bicuculline
CA	Cornu Ammonis
CAMKI	Ca2+/calmodulin-dependent protein kinase I
CaMKII	Ca^{2+}/calmodulin-dependent protein kinase II
cAMP	Cyclic Adenosine Monophosphate
CC	Chelerythrine Chloride
cDNA	Complementary DNA
cGK	cGMP-dependent Protein Kinase
CI-AMPAR	Ca^{2+}-Impermeable AMPAR
cKO	Conditional Knockout
CNIH2/3	Cornichon Family AMPA Receptor Auxiliary Proteins 2/3
CNS	Central Nervous System
CP-AMPAR	Ca^{2+}-Permeable AMPAR
CPG2	Candidate Plasticity Gene 2
CPG55845	CPG 55845 Hydrochloride
CREB	cAMP-response element binding protein
CSF	Cerebrospinal Fluid
CTD	Carboxy-terminal Domain
CV	Coefficient of Variation
DA	Dopamine
DAG	Diacylglycerol
DAPI	4′,6-diamidino-2-phenylindole
DG	Dentate Gyrus
DIV	Days in Vitro
DLGAP1	Discs Large Homologue-Associated Protein 1
DM	Dissection Media
DNA	Deoxyribonucleic acid
Dnm2	Dynamin-2
DS	Down's Syndrome
DTT	Dithiothreitol
ECS	electroconvulsive stimulation
eEF2K	eukaryotic elongation factor 2 kinase
Endo2/3	Endophilin-2/3
EPSC	Excitatory Postsynaptic Current
EPSP	Excitatory Postsynaptic Potential
ER	Endoplasmic Reticulum
ERG-1	Early Growth Response Protein
EV	Extracellular Vesicles
fAD	Familial Alzheimer's Disease
FUNCAT	Fluorescent Non-Canonical Amino Acid Labelling
FV	Fibre Volley
GABA	Gamma Aminobutyric Acid
GFAP	Glial fibrillary acidic protein
GFBD	Growth Factor Binding Domain

GKAP	Guanylate Kinase-Associated Proteins
Gl	Glycosylation
GRIP1/2	Glutamate Receptor-Interacting Protein 1/2
H89	H-89 Dihydrochloride
HBD	Heparin-Binding Domain
HEK	Human Embryonic Kidney cells
HFS	High-Frequency Stimulation
IEG	Immediate Early Gene
IEM-1460	N,N,N-trimethyl-5-[(tricyclo[3.3.1.13,7]dec-1-ylmethyl)amino]-1-pentanaminium bromide hydrobromide
IntDen	Integrated Density
IPSC	Induced Pluripotent Stem Cell
IS	Interneuron Specific
IS	Interneuron-specific
JMR	Juxtamembrane Region
KO	Knockout
LBD	Ligand Binding Domain
LFS	Low-Frequency Stimulation
LTD	Long-Term Depression
LTP	Long-Term Potentiation
mAchR	Muscarinic Acetylcholine Receptor
MAGUK	Membrane Associate Guanylate Kinases
MAP2	Microtubule Associated Protein 2
MAPK	Mitogen activated protein kinase
MCPG	± A-Methyl-4-Carboxyphenylglycine
MEM	Minimal Essential Media
mGluR	Metabotropic Glutamate Receptor
MQ	Milli-Q
mRNA	Messenger Ribonucleic Acid
mTOR	Mammalian Target of Rapamycin
MW	Molecular Weight
NaF	Sodium Fluoride
NES	Nuclear Export Signal
NFκB	Nuclear Factor-Kappa B
NGM	Normal Growth Media
Ni	Nitrosylation
NLS	Nuclear Localization Signal
NMDAR	N-Methyl- d-aspartate
NO	Nitric Oxide
NOS	Nitric Oxide Synthase
NRD	Nuclear Retention Domain
NSF	N-ethylmaleimide sensitive fusion protein
NTD	N-Terminal Domain
ODN	Oligodeoxynucleotide
PAK3	P21-Activated Kinase 3
PBS-MCS	Phosphate Buffered Saline- $MgCl_2$ $CaCl_2$ & Sucrose
PC1	Protein Convertase 1
PCL	Pyramidal Cell Layer
pCREB	Phosphorylated cAMP-response element binding protein
PD	Postnatal Day
PDE5	Phosphodiesterase 5
PDL	Poly-D-Lysine
PDZ	PSD-95/Drosophilla discs large/zona occludens-1
PI3K	Phosphoinositide 3-Kinase
PICK1	Protein Interacting With PRKCA 1
PKA	Protein Kinase A
PKB	Protein Kinase B
PKC	Protein Kinase C
PKD	Protein Kinase D
PKG	Protein Kinase G
PKM	Protein Kinase M
PKMζ	Protein Kinase M-Zeta
PKN	Protein Kinase N
PLA	Proximity Ligase Assay
PLC- γ	Phospholipase C-Gamma
PML	Promolocytic Leukaemia
PMSF	Phenylmethylsulfonyl Fluoride
PP1	Protein Phosphatase 1
Prox1	Prospero-Related Homeobox 1
PRP	Plasticity Related Proteins
pS	Phosphoserine
PS1	Presenilin-1
PSD	Post-Synaptic Density
pT	Phosphothreonine
PTM	Post-Translational Modification
PTM	Posttranslational Modifications
pY	Phosphotyrosine
RI	Rectification Index
RNA	Ribonucleic acid
RT	Room-temperature
RT-qPCR	Reverse Transcription Quantitative Polymerase Chain Reaction
sAD	Sporadic Alzheimer's Disease
SAP97	Synapse-associated protein 97

SAPAP1	SAP90, PSD-95-associated protein 1
sAPPα	Secreted Amyloid Precursor Protein Alpha
sAPPβ	Secreted Amyloid Precursor Protein Beta
sAPPη	Secreted Amyloid Precursor Protein Eta
SC	Schaffer Collateral
SEM	Standard Error of the Mean
sGC	Soluble Guanylyl Cyclase
siRNA	Small interfering RNA
STD	Short-Term Depression
STP	Short-Term Plasticity
SUMO	SUMOylated
TARP	Transmembrane AMPA Receptor Regulatory Proteins
TBI	Traumatic Brain Injury
TBS	Theta Burst Stimulation
TBTA	Tris (1-benzyl-1H-1,2,3-triazol-4-yl)methyl) amine
TCEP	Tris(2-carboxyethyl)phosphine hydro-chloride
THC	Δ(9)-tetrahydrocannabinol
TLE	Temporal Lobe Epilepsy
TMD	Transmembrane Domain
TNF-α	Tumour Necrosis Factor-Alpha
TrkB	tropomyosin receptor kinase B
TTX	Tetrodotoxin
Ub	Ubiqutination
WT	Wild-Type
α7nAChR	α7 nicotinic acetylcholine receptors
αBGT	α-Bungarotoxin

Chapter 1:
Controlling Plasticity with Molecules and What This Means for Memory and Disease: Mediators, Messengers, Modulators and More

1.1. Learning and Memory

Learning and memory are fundamental phenomena which describe the ability of both animals and humans to acquire, retain, and retrieve information (Seyfarth and Cheney, 2003). Many of these processes consist of mechanisms necessary to permit the recognition of the familiar, prediction of future events, and assessment of behaviour, allowing humans and animals to make sense of the environment around them (Sherry and Schacter, 1987; Camina and Güell, 2017). The formation, storage, and retrieval of both short- and long-term memories is understood to occur through cellular changes within the central nervous system (CNS), contributing to specific modifications in the strength and efficacy of neuronal cells to respond to stimulation (Okano, 2000). This idea was first proposed by American psychologist William James, who linked the properties of plasticity with those of behavioural habits (James, 2007). Here, William James proposes;

> *"When two elementary brain-processes have been active together or in immediate succession, one of them, on reoccurring, tends to propagate its excitement into the other."* (James, 1890)

The idea that the nervous system has intrinsic plastic properties capable of reshaping the relationships between neurons was later supported by descriptions, both philosophical and anatomical, of the synapse. The synapse was first described by Italian neuropsychiatrist Eugenio Tanzi (Tanzi, 1893) as *"minimal interruptions between adjacent and functionally related neurons"* through which activity *"increases the nutritional processes… accompanied by hypertrophy"* and effectively decreases the *"distance between adjoining and contiguous neurons"*. This proposition was further expanded upon by Tanzi's disciple Ernesto Lugaro, who further suggested that repetition or habitual processes *"establish reciprocal connections"* and *"facilitates the transmission of functional activity from one to the other"* (Lugaro, 1898). These ideas were further supported by anatomical observations made by Italian biologist Camillo Golgi and Spanish pathologist Santiago Cajal (Golgi, 1885; Cajal, 1893; Cajal, 1909), who provided detailed descriptions of nerve cells as well the appearance of *"thorns or short spines"* (Cajal, 1888; Cajal, 1896), now considered the first observations of synapses (Berlucchi and Buchtel, 2009; Yuste, 2015).

The early concepts describing the plastic abilities of the nervous system, together with direct observations of neurons and the connections between them, was later refined into a concrete model of synaptic plasticity by Canadian psychologist Donald Hebb (Hebb, 1949). Simply put, Hebb describes synaptic plasticity as a mechanism through which repeated and persistent firing of a presynaptic cell, which leads to the firing of a consecutive postsynaptic cell, will increase the strength, stability, and efficacy of the connection, through a form of growth or metabolic change within the cell. This model has become to be known as Hebbian plasticity and remains one of the leading theories on how neural tissue adapts to change, and may contribute, in part, to the underlying processes of learning and the formation of long-term memories (Abbott and Nelson, 2000).

1.1.1. *The Hippocampus*

Observations of the mechanisms and functions of learning and memory have been made since as early as the late 19th century (Ribot, 1882; Freud and Breuer, 1895; Kennedy, 1898; Bregman, 1899; Barr and Bieliauskas, 2016). Around this time, Ebbinghaus, (1885) first introduced the division of short-term and long-term memory, while Ribot, (1882) began to describe the necessary conditions for memory (*"the conservation of certain conditions, their reproduction, and their localization in the past"*), beginning to distinguish between 'temporary amnesia,' 'periodical amnesia,' and 'progressive amnesia'. However, many of these observations did so in the absence of references to the nervous system. The early 20th century bought with it observations of the mechanisms which may permit formation or maintenance of memories; including the pairing of stimuli in classical conditioning (Semon, 1911; Pavlov and Gantt, 1928), the importance of emotion in memory strength (Gregor, 1907), and the acquisition of mnemonic memories (Ranschburg, 1911). Importantly, these observations aided in cytoarchitectural (Brodmann, 1909) and physiological mapping of the cerebral cortex (Silverstein, 2012), and identification of a region-specific, functional representation of the entire body (Penfield and Boldrey, 1937). These observations were further characterised by limb amputations and attributable changes to cortical and peripheral sensitivity (Katz, 1920; Haber, 1955; 1958), which began to both localise brain function to anatomy as well as indicate that experience-dependent changes may correlate to changes in anatomy and physiology.

Following these findings, observations began to arise providing evidence towards the localization of memory formation specifically. Zingerle, (1912) first described marked memory disturbances and reduced intelligence in a 40-year-old patient in which the right temporal lobe was absent. Following this, Gamper (1928) and Grünthal (1947) described clear diencephalic damage and impaired memory related to alcohol-related brain damage and hypoglycaemia, respectively.

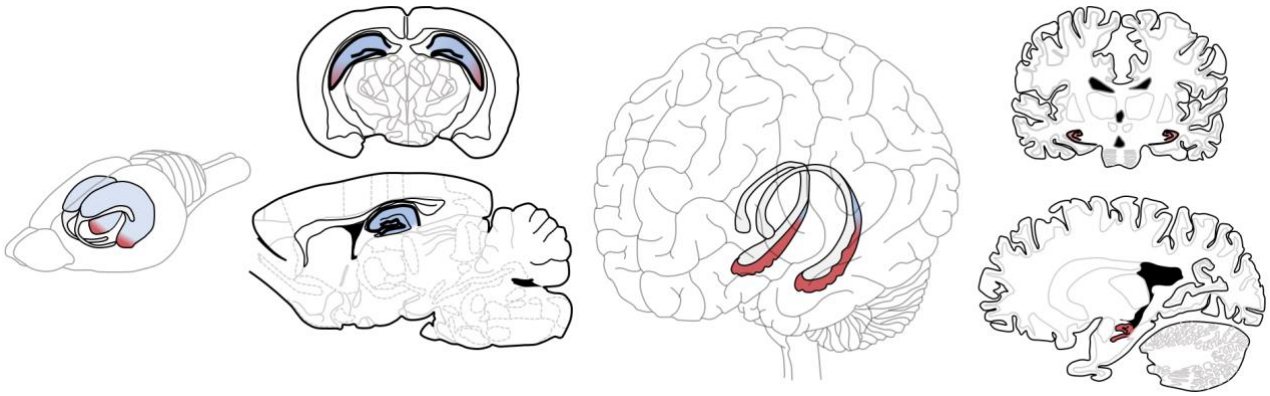


Figure 1-1 | Homologous regions of the hippocampus in the human and rat brains. Representative images show rat brain (left) and dorsal (■) and ventral (■) hippocampus, with coronal (top left) and sagittal (bottom left) sections. Right images show stylized human brain (right), and dorsal (■) and ventral (■) hippocampus, with coronal (top right) and sagittal (bottom right) sections.

Most recently, a well-known case of this involves patient Henry Molaison (H.M.) who suffered from major seizures following an accident in his childhood. In an effort to resolve this, H.M. underwent surgery for a bilateral medial temporal lobectomy, removing the hippocampus, amygdala, and the adjacent parahippocampal gyri (Squire, 2009). Following surgery, patient H.M. experienced profound impairments in his ability to form long-term declarative memories,

> *"so severe so as to prevent [H.M.] from remembering the location of the rooms in which he lives, the names of his close associates, or even the way to the toilet"*
> (Scoville, 1954)

leading neurosurgeon William Scoville to conclude:

> *"…that the anterior hippocampus and hippocampal gyrus, either separately or together, are critically concerned in the retention of current experience"*
> (Scoville and Milner, 1957)

Over the next decade, neuroanatomical studies began to evaluate the intricate structure and function of the medial temporal lobe memory system and its relationship to the central nervous system (Squire and Zola-Morgan, 1991).

Encompassing the hippocampus, in addition to associated entorhinal, perirhinal, and parahippocampal cortices, the medial temporal lobe memory system has come to be understood to

be responsible for the formation and consolidation of long-term memories, but ultimately is not an absolute storage structure as Scoville may have implied (Penfield and Milner, 1958; Marslen-Wilson and Teuber, 1975; Steinvorth et al., 2005; Kirwan et al., 2008).

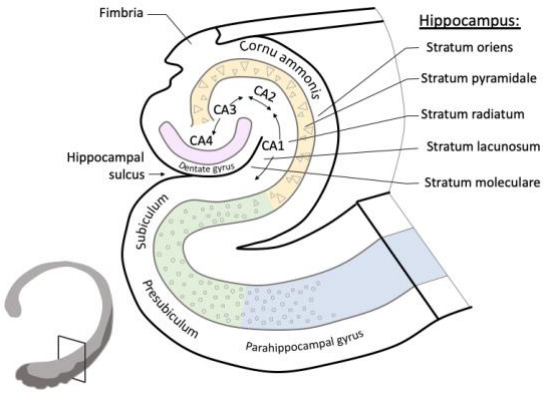


Figure 1-2 | Schematic diagram of the Hippocampal formation. Coronal cross-section (as indicated bottom left) of the hippocampus shows subfields, including the dentate gyrus ( ), cornu ammonis ( ), entorhinal cortex ( ), and parahippocampal gyrus ( ).

Because of its elegant structure and very ordered cellular connections, the hippocampus has become a popular practice to examine underlying mechanisms of memory through electrophysiological, cellular, and molecular neurobiology practices. Early and ongoing experimental practices have utilized non-human animals as a means of investigating the underlying mechanisms and behaviours which may drive many neurodegenerative, cognitive and affective disorders in human beings. Primarily, rodent models (especially *Mus musculus* and *Rattus norvegicus*) have been the most widely used models in biomedical research for many years, encompassing approximately 20% in the 1970s and 1980s to around 50% of neuroscience-related research in recent years (Ellenbroek and Youn, 2016). Arguably, the use of rodents has formed a strong basis for preclinical research regarding drug therapies, genome-editing technologies, and a means to investigate physiological, anatomical, biochemical and pharmacological underpinnings of neurodegenerative disorders. The anatomy of the rat cortex and hippocampus has been thoroughly characterised in order for comparisons to human be made. These comparisons allow the identification of possible anatomical, functional, and behavioural linkages. As shown by Figure 1-1., the gross anatomy of the hippocampus differs substantially. While the curved, elongated structure is constant throughout all mammals (Strange et al., 2014), the location and extent of the hippocampal boundaries differ with respect to the dorsoventral axis. The primate hippocampus is primarily contained within the temporal lobe, while much of the rodent hippocampus rests dorsally beneath the corpus callosum (Insausti, 1993; Royer et al., 2010; Lisman et al., 2017). Importantly, the overall pattern of connectivity shows a high degree of conservation across mammals, such that while the gross anatomy may differ, the fields of the hippocampal formation are linked by similar and largely unidirectional connections.

The hippocampus itself is subdivided into the Cornu Ammonis (CA), also referred to as CA1–4, and the dentate gyrus (DG; Figure 1-2). Parahippocampal and entorhinal cortices project afferents into the dentate gyrus via the perforant path (Burwell and Amaral, 1998; Wible, 2013). Here, afferent

axons terminate on the granule cells of the dentate gyrus which in turn project mossy fiber tracts to the CA3 field of the hippocampus. Here, pyramidal cells of area CA3 send collateralized axons of the Schaffer collaterals, the major input to CA1. CA1 provides the major output of the hippocampus, sending efferent projections to the subiculum which in turn projects to many cortical and subcortical targets (Witter and Groenewegen, 1990; O'Mara et al., 2001). This conserved hierarchy of connectivity has led to the proposal that he hippocampus may serve as a central node of rapid synaptic change, such that information is processed through a limited number of synaptic changes to be gradually interleaved into existing networks of knowledge within the cortex (McClelland et al., 1995).

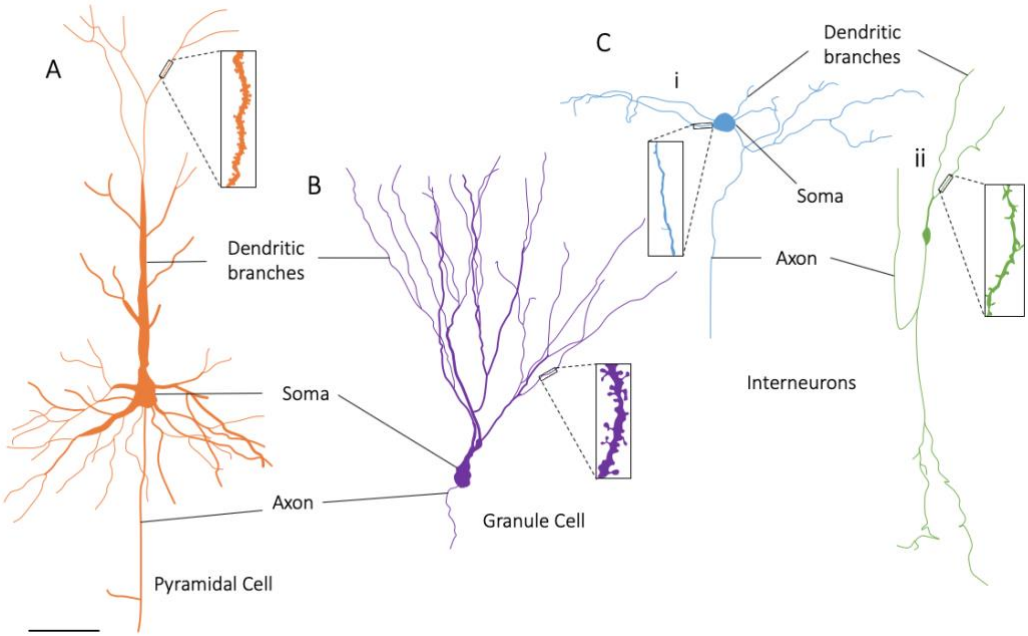


Figure 1-3 | Principal cells of the hippocampus. Morphological characteristics of A) CA1-3 pyramidal neurons (■), B) dentate gyrus granule cells (■), and C) i. basket (■), and ii. interneuron-specific (IS, ■) interneurons. Inset image shows representative magnification of pyramidal, granule, basket cell and bipolar spines. Scale bar = 50 μM.

As mentioned above, primary cells of the hippocampus include glutamatergic granule and pyramidal cells of the DG and CA1–4 areas, respectively. As shown by Figure 1-3., pyramidal cells can be defined by their large pyramidal-shaped soma (approximately 20 μm; Figure 1-3A) and distinct apical and basal dendritic arborization. Alternatively, granule cells of the DG are defined by their small soma (approximately 10 μm; Figure 1-3B) and unique apical, cone-shaped arborization (Amaral et al., 2007), receiving information from the perforant path, and projecting mossy fiber axons towards CA3. The dendrites of both pyramidal and granule cells are the sites of small (< 1 μm) protrusions called

spines, and are the site of the postsynaptic response (Nimchinsky et al., 2002; Bekkers, 2011). In addition to the excitatory principal cells, the hippocampus also contains subpopulations of interneurons, primarily expressing the inhibitory transmitter γ-aminobutyric acid (GABA; Figure 1-3C). The term interneuron conveys the unifying principal that these inhibitory cells regulate local circuit excitation, and display diverse morphological and chemical properties. Specific types of interneurons are classified based on their clear axonal or dendritic processes, such as basket cells (Figure 1-3 Ci.), which form dense connections on the soma of pyramidal cells (Fasano et al., 2017). In addition, various populations of interneurons were found to contain different peptides (e.g. parvalbumin, cholecystokinin, somatostatin, calbindin and calretinin), resulting in additional classifications based on neurochemical composition (Freund, and Buzsáki, 1996). While the expression of parvalbumin, calbindin and calretinin appears to identify specific groups of morphologically distinct and input-specific interneurons, some neurochemical markers overlap and have been found to co-exist within distinct population-specific, morphologically distinct populations (Kosaka et la., 1987; Losonczy et al., 2002), as well as neurochemically distinct populations of morphologically identical interneurons (e.g. parvalbumin- and cholecystokinin-positive basket cells; Fasano et al., 2017). Finally, populations have been identified based on their functional characteristics, such as interneuron-specific (IS) cells (Figure 1-3 Cii), which provide inhibitory feedback of other interneurons (Chamberland and Topolnik, 2012), and oriens-lacunosum moleculare (OLM) interneurons, which preferentially innervate CA1 pyramidal neuron dendrites (Müller and Remy, 2014; Maccaferri and Lacaille, 2003).

1.2. Mechanisms of Cellular Memory

1.2.1. *Long Term Potentiation*

Following decades of research, a canonical model has emerged, which permits observation and quantification of Hebbian plasticity following electrical or chemical stimulation. This model was initially described by Tim Bliss and Terje Lømo with work done in the dentate gyrus of the hippocampus of anaesthetized (Bliss and Lomo, 1973) and unanaesthetized (Bliss and Gardner-Medwin, 1973) rabbits. As shown by Figure 1-4., tetanisation of the perforant path by high frequency simulation (HFS) produced a persistent increase in the efficacy of the excitatory postsynaptic potential (EPSP) to subsequent stimulations, specific to the tetanized pathway. This increase was found to last the length of the experiment in anaesthetized rabbits, but was found to slowly decay to baseline across 3 days in unanaesthetized rabbits (with one exception, still potentiated following 16 weeks). This long-lasting enhancement in the synaptic response was later termed long-term potentiation (LTP) and has become a principal experimental method and theoretical model for examining the processes and underlying mechanisms of Hebbian plasticity in neural tissue (Lomo, 2003). For Hebbian plasticity to serve as a mechanism through which cellular correlates of memory occur, four properties have been described which may permit this. Firstly, as described by Tim Bliss and Terje Lømo's early experiments, LTP exhibits *input specificity*, such that potentiation induced at individual synapses only affects those which contribute directly to LTP and does not spread to non-potentiated synapses. This has been proposed to occur through Frey and Morris' mode of 'synaptic tag and capture' (Dynes and Steward, 2007), wherein currently unidentified proteins or processes act to target plasticity related proteins (PRP) exclusively to activated or potentiated synapses. Secondly, LTP displays rules of *associativity*, such that weakly activated synapses can be strengthened by a strong stimulus at spatially distinct synapses (Shors and Matzel, 1997). Thirdly, and similar to associativity is *cooperativity*: LTP can be induced by either a strong stimulation or in the case of cooperative potentiation,

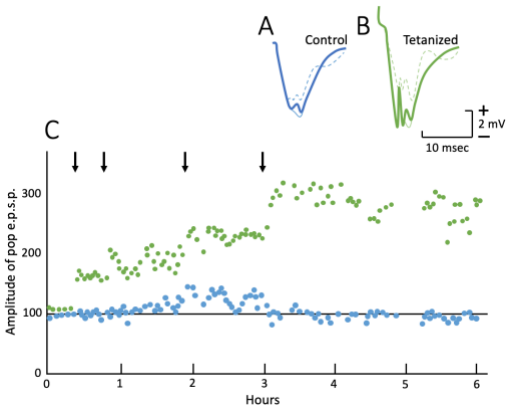


Figure 1-4 | The discovery of LTP. Long term potentiation was first reported in the perforant path in anaesthetized rabbits. Responses from both control and tetanized pathways A) before and B) after conditioning. C) Graph showing the amplitude of the population EPSP for control (●) and tetanized (●) pathways. Conditioning trains (arrows) were given at a rate of 15/sec for 10 sec.

many individual weak stimulations converging spatially and temporally at one pathway, neuron, or synapse may act to collectively depolarize the postsynaptic membrane enough to produce LTP (Kitajima and Hara, 1991). Lastly, and perhaps the most distinct characteristic of LTP is its *persistence*, that is the ability of LTP to last from several minutes to many months (Abraham, 2003), and it is this that distinguishes LTP from other forms of plasticity expressed on much shorter timescales (milliseconds to minutes), such as paired-pulse facilitation, and post-tetanic potentiation (Citri and Malenka, 2008).

Since 1973, the field has expanded, covering many of the plausible aspects that might contribute to the expression and persistence of Hebbian plasticity using LTP as a model, examining electrophysiology, molecular biology, biochemistry, genetics and epigenetics (Lynch et al., 1979; Teyler, 1999; Matynia et al., 2002). Early observations began to describe underlying mechanisms governing LTP. Of these, it was shown that learning paradigms were correlated with an increase in protein synthesis and preventing this process impaired the persistence and consolidation of memories (Flexner et al., 1962; Flexner et al., 1963; Agranoff and Klinger, 1964; Agranoff et al., 1965; Flexner et al., 1965; Flexner et al., 1966; Agranoff, 1967; Shashoua, 1976). Following this, the cellular correlate of protein synthesis-dependent memory consolidation was sought. In 1984, Manfred Krug showed that the maintenance of hippocampal dentate gyrus LTP beyond 3 hours *in vivo* could be blocked by application of the protein synthesis inhibitor anisomycin (Krug et al., 1984). Various researchers later replicated these findings, observing similar results throughout the hippocampus using a variety of stimulation methods (Frey et al., 1988; Frey et al., 1989; Otani and Abraham, 1989; Fazeli et al., 1993; Osten et al., 1996; Nayak et al., 1998). Through these observations, it was theorized that LTP displays distinct phases, depending on the necessity of protein synthesis. Short-term plasticity (STP) is defined as a form of plasticity which decays rapidly, lasting approximately 20–40 minutes, and is thought to be dependent on primarily presynaptic mechanisms (Hennig, 2013), including; residual presynaptic calcium (Katz and Miledi, 1968; Kamiya and Zucker, 1994; Wu and Saggau, 1994), saturation or dislocation of presynaptic calcium buffers (Caillard et al., 2000; Blatow et al., 2003; Timofeeva and Volynski, 2015), enhancement of presynaptic calcium currents (Borst and Sakmann, 1998; Leal and Klein, 2009), and changes in the sensitivity of calcium sensors such as synaptotagmins, controlling delayed versus fast presynaptic neurotransmitter vesicle release (Burgoyne and Weiss, 2001; Stevens and Sullivan, 2003; Burgoyne, 2004; Yoshihara and Montana, 2004; Jackman et al., 2016; Figure 1-5).

Early phase (E-LTP or LTP1) and late-phase (L-LTP or LTP2) LTP are further distinguished by their dependency on protein synthesis. LTP1 is thought to last anywhere from one to many hours and is independent of protein synthesis (Krug et al., 1984), emerging through post-translational

modifications of pre-existing proteins by calcium-dependent enzymes, as well as expansion of the spine cytoskeleton and glutamate receptor trafficking (Blundon and Zakharenko, 2008; Bosch et al., 2014; Park, 2018b; Figure 1-5). LTP2 on the other hand, is invariably long-lasting, persisting for up to many hours post-stimulation (Malenka, 1991a; Schulz and Fitzgibbons, 1997; Frey et al., 2001; Figure 1-5). Evidence suggests that LTP2 is dependent on the dendritic synthesis of PRPs, for its persistence. This 'local protein synthesis' comes from studies utilizing dendrites isolated from the soma, and thus preventing distal trafficking of new proteins. In these preparations, L-LTP could still be produced and was blocked by the application of protein synthesis inhibitors (Manahan-Vaughan et al., 2000; Cracco et al., 2005; Huang and Kandel, 2005; Vickers and Wyllie, 2007). These observations likely indicate a role in the local translation of pre-existing mRNA present at or near the activated synapses (Steward and Schuman, 2001), likely in order to engage mechanisms which consolidate the changes initiated during LTP1 (Hardt et al., 2013). There is increasing evidence that the consolidation of the synaptic changes during LTP2 occur, in part, through the synthesis and function of neuromodulators, such as dopamine (Frey et al., 1991a; Frey et al., 1991b; Huang and Kandel, 1995; Granado et al., 2008), BDNF (Ying et al., 2002; Lu et al., 2008; Panja and Bramham, 2014), and serotonin (Mlinar et al., 2015). Furthermore, evidence exists of an additional phase of LTP (LTP3) which requires the transcription of new mRNA (Nguyen et al., 1994; Frey et al., 1996; Vickers et al., 2005; Ryan et al., 2011), and has been described to underlie the persistence of long-term memories, lasting anywhere from hours (Nguyen et al., 1994; Frey et al., 1996) to months (Abraham et al., 2002). These gene transcripts may underlie mechanisms which expand the pool of newly synthesised proteins, replenish proteins and mRNA directly involved in early enhancements of LTP (Abraham and Williams, 2003), as well as orchestrate chromatin and epigenetic modifications (Alberini and Kandel, 2014).

As the expression characteristics of LTP were slowly being elucidated, a second debate arose within the field — that which was concerned with the mechanistic locus of LTP. For many years the debate sat split down the centre of the synaptic cleft. On one side, those believing presynaptic changes underlie the increased synaptic response, and on the other; those who believe postsynaptic changes dictate changes in potentiation. Early studies found evidence for the presynaptic component of LTP through increases in neurotransmitter release probability (Bekkers and Stevens, 1990; Malinow and Tsien, 1990), the number of active release sites (Bolshakov et al., 1997), or the quantal size of neurotransmitter vesicles (Kullmann and Nicoll, 1992; Stricker et al., 1996). Countering this, the postsynaptic argument posited that changes in the synaptic expression of N-Methyl-d-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are responsible for the observed increase in the EPSP, as evidenced from increases in the AMPA:NMDA

current ratio (Watt et al., 2004; Sjostrom et al., 2007), the number of AMPAR (Hayashi et al., 2000) and NMDAR (Berretta et al., 1991; Xie et al., 1992), the dependence on AMPAR expression (Zamanillo et al., 1999; Granger et al., 2013), as well as changes in structural plasticity (Matsuzaki et al., 2004).

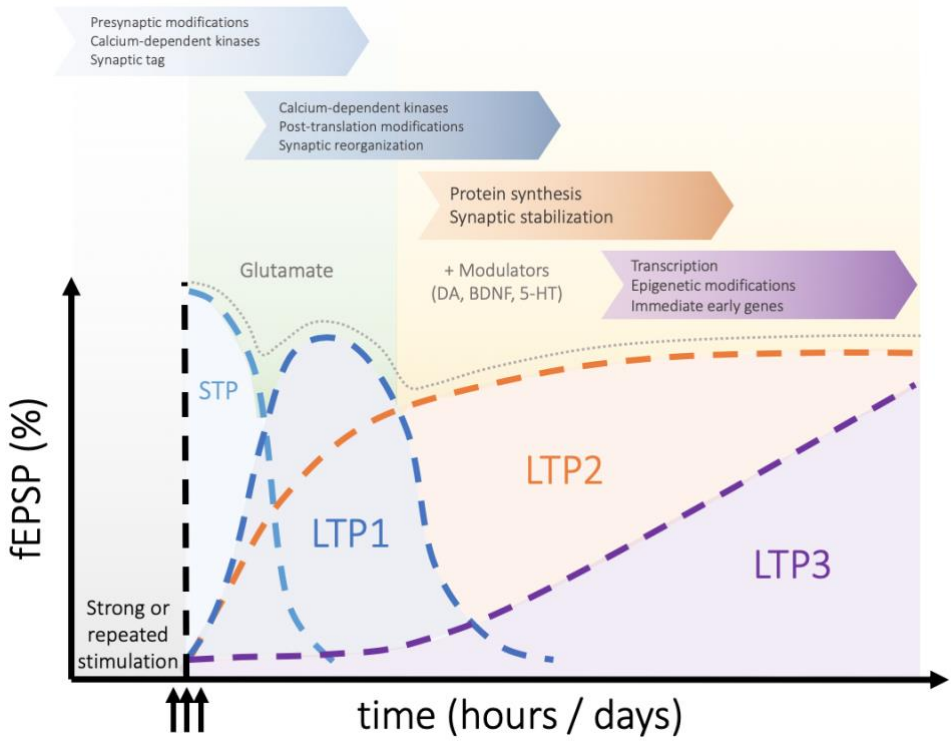


Figure 1-5 | Schematic illustration of separate phases of synaptic long-term potentiation. STP (■) is defined by a short-lasting potentiation of the EPSP induced by weak tetanisation. LTP1 (E-LTP, ■) is defined primarily by a sensitivity to kinase inhibitors, as well as increases in presynaptic quanta and requires strong tetanisation, decaying within an hour. LTP2 (L-LTP, ■) is a long-lasting potentiation which is sensitive to protein-synthesis inhibitors and additive to LTP1, lasting > 3 hours. LTP3 (■) is dependent on new gene transcription and is sensitive to inhibitors of this. LTP2 and LTP3 can be achieved through strong or repeated tetanization or addition of modulators including neurotrophins or growth factors including dopamine (DA), brain-derived neurotrophic factor (BDNF), and serotonin (5-HT). Originally defined by Reyman & Frey, (2007).

Importantly, throughout this debate, a proportion of researchers began to shift their focus to phenomena of 'silent synapses'. These synapses are termed as such due to the absence of functional AMPAR during baseline responses, able to be 'awoken' or 'unsilenced' upon LTP stimulation through an NMDAR-dependent mechanism. Similar to the debate surrounding the locus of LTP expression, silent synapses sparked a parallel debate regarding the mechanism of synapse unsilencing. Many of those on the presynaptic side of the debate carry their beliefs to the unsilencing of silent synapses, such that these synapses can be explained primarily through low release probability of glutamate (Gasparini et al., 2000; Montgomery et al., 2001). Likewise, with LTP, postsynaptic mechanisms may also explain a mechanism to unsilence silent synapses. Initial studies posited that unsilencing occurs

throughout development, with many synapses initially containing NMDAR but little to no AMPAR, finding that miniature EPSCs (mEPSC) frequency increases throughout development – concluding that AMPAR insertion underlies synapse awakening (Petralia et al., 1999; Vincent-Lamarre et al., 2018; Wright et al., 2020; Xu et al., 2020). Further, evidence suggests that LTP underlies one mechanism through which silent synapses are awoken (Liao et al., 1995; Durand et al., 1996; Liao et al., 1999).

1.2.2. *Long-Term Depression*

Early studies investigating mechanisms that serve to increase the strength between neural connections (such as LTP) theorized that a system such as this must have a way to actively *decrease* synaptic strength in contrast. The reversible nature of LTP, such that LTP undergoes passive decay following extended potentiation over a period of days to weeks, could theoretically be sufficient to employ a mechanism of information storage. Yet, a system serving to only actively increase the strength of a synapse would inevitably reach a point of saturation of maximal information storage. Thus, the ability to actively decrease and reset individual synaptic weights adds a distinct computational advantage. Therefore, long-term depression (LTD) became the focus of many studies in the 80s and 90s as a mechanism which may act to 1) prevent the saturation of LTP (Byrne, 2010), 2) act as an active resetting of previously potentiated synapses (Barr et al., 1995; Muller et al., 1995; Villarreal et al., 2002), 3) amplify the signal-to-noise ratio of neighbouring potentiated synapses (Dayan and Willshaw, 1991; Kemp and Manahan-Vaughan, 2007), or 4) mediate mechanisms underlying forgetting (Tsumoto, 1993; Nabavi et al., 2014).

The earliest publications describing what we now understand to be LTD, described the effect of low frequency stimulation (LFS) on the postsynaptic response (Barrionuevo et al., 1980; Levy and Steward, 1983; Lynch et al., 1976; Lynch et al., 1977). Of note is Barrionuevo & Lynch (1980) who elicited LFS on both control (non-potentiated) and previously potentiated Schaffer collateral–Commissural projections in the rat hippocampus. Interestingly, they describe a persistent depression of both experimental groups, lasting 15–30 minutes following LFS, concluding LTD to be a mechanism by which the hippocampus employs to both offset enhancement by LTP and decrease the baseline strength of synapses (Figure 1-6). While it is argued that the depression of the previously potentiated pathway may in fact be depotentiation, a mechanism distinct from LTD (Huang and Hsu, 2001; Sanderson, 2012), the effect of LFS on control synapses was evident.

Following these findings, efforts were made to understand the mechanisms behind LTD. Dudek and Bear, (1992) first examined LTD under the principals previously applied to LTP, finding that LTD in the hippocampus shows *input specificity*, *cooperativity*, *associativity*, and *persistence*. These findings, and many more describe a similar mechanism to LTP. It has been found that the induction of LTD 1) is dependent on the simulation protocol, such that 900 pulses at 1-3Hz consistently yielded a depression of the EPSP, while 10Hz produced no change in EPSP and 50Hz caused potentiation (Dudek and Bear, 1992; Figure 1-6), 2) is blocked by an NMDA receptor antagonist (Mulkey and Malenka, 1992), 3) does not prevent later LTP by HFS, 4) reaches saturation, or maximal depression (Levy and Steward, 1983; Christie and Abraham, 1992), and 5) can be reversed by LTP (Abraham and Goddard, 1983). These discoveries generated a new outlook on the modification of synaptic information processing through controlled bidirectional changes in synaptic strength, defining a mechanism separate from LTP, produced through opposing stimulation patterns, yet converging through the necessity of the NMDA receptor.

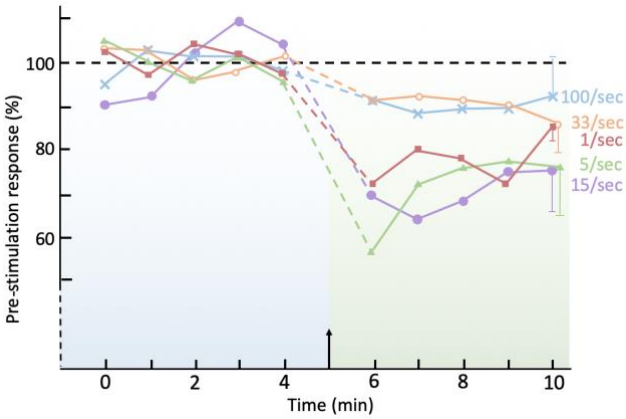


Figure 1-6 | The discovery of LTD. The effect of low frequency stimulation (arrow) on the population spike amplitude. 1/sec (■), 5/sec (▲), 15/sec (●), 33/sec (○), 100/sec (×). Data from Barrionuevo et al., (1980) | The discovery of LTD.

As observed with LTP, the induction and persistence of LTD may be separated into distinct, overlapping mechanisms. Short-term depression (STD) is a transient form of synaptic depression shown to occur following trains of frequent stimulation. This STD is thought to occur through, 1) a depletion of the readily releasable pool (RRP) of neurotransmitter vesicles at the presynaptic terminal (Zucker and Regehr, 2002; Rizzoli and Betz, 2005; Regehr, 2012), 2) inactivation or saturation of vesicle fusion sites (Neher and Sakaba, 2008; Hosoi et al., 2009), 3) reduction in presynaptic calcium currents (Forsythe et al., 1998; Xu and Wu, 2005; Catterall and Few, 2008; Neher and Sakaba, 2008; Hosoi et al., 2009), and 4) desensitization of postsynaptic glutamate receptors (Antzoulatos et al., 2003; Koike-Tani et al., 2008). Similar to LTP, initial short-lasting changes such as STD, have the capacity to evolve into stronger, more persistent forms. As first shown by Barrionuevo et al., (1980) and later by Dudek and Bear, (1992b), decreasing the rate at which synapses are stimulated, greatly enhanced the level of synaptic depression observed, such that 5-10 Hz stimulation results in a short lasting LTD, “decaying” back to baseline (E-LTD), while lower 1-3 Hz stimulation results in long-lasting depression of synaptic depression (L-LTD; Dudek and Bear, 1992b; Kauderer and Kandel, 2000).

Importantly, as with early-phase LTP, both STD and E-LTD have been shown to be protein synthesis-independent, with the expression of E-LTD likely involving the activity of protein phosphatases (Lisman, 1989; Mulkey et al., 1993). Conversely, long-lasting L-LTD has been shown to involve an additional protein synthesis-dependent component, necessary for its maintenance (Huber et al., 2000; Kauderer and Kandel, 2000; Sajikumar and Frey, 2004), for at least 4-5 hours following induction *in vivo.* Interestingly, the persistence of L-LTP may depend on pre-existing mRNA for local translation of required proteins (Huber et al., 2000; Sajikumar and Frey, 2003). Beyond this, some evidence also exists for an additional requirement of transcription ex *vitro* (Lindecke et al., 2006) and a much later (> 24 hours) role *in vivo* (Kemp et al., 2013).

Importantly, while LTP involves the formation, growth, and maturation of dendritic spines and synapses, LTD may govern an opposing mechanism of synapse shrinkage and elimination. Application of NMDA to cultured neurons showed a protein phosphatase-, protein synthesis- and transcription-dependent reduction in the number of functional synapses and spines (Ramiro-Cortés and Israely, 2013; Henson et al., 2017), while LTD-inducing stimulation was found to shrink spines (Nägerl et al., 2004), in a manner which could be rescued by LTP-inducing stimulation (Zhou et al., 2004). These results have been further corroborated by *in vivo* studies, in which induction of LTD eliminated both depressed synapses, as well as weakly-integrated neighbouring synapses (Wiegert and Oertner, 2013). These results indicate that LTP- and LTD-like mechanisms promote bidirectional changes in both physiological as well as morphology to regulate the expression of plasticity.

1.2.3. *Homeostatic plasticity*

As mentioned above, Hebbian plasticity in the central nervous system acts to encode information through changes in the strength of synaptic connections. However, a caveat of this means stronger synapses are more likely to depolarize the postsynaptic neuron, increasing the probability that they will undergo further potentiation. A system such as this, without forces that prevent uncontrolled excitability, would lead to unconstrained synaptic strengthening, wherein the specificity of information breaks down and can no longer be effectively stored through differences in synaptic strength (Whitt et al., 2014). In theory, without a mechanism to maintain relative differences between competing inputs, a mechanism such as LTP, which aims to increase the excitability of cells or synapses, would only serve to further potentiate other inputs, increasing the probability of pre- and postsynaptic associations. Therefore, homeostatic plasticity allows for the adjustment of neuronal or synaptic weights, in order to avoid hyperexcitability, maintain the ability of inputs to remain plastic, and prevent the loss of valuable information following both strong synaptic activity or

sustained periods of inactivity. Here, relative strengths are maintained within a physiological range for Hebbian plasticity to occur (Figure 1-7A.)

There are several ways in which neurons may compensate for periods of increased or decreased activity. These include altering their intrinsic excitability (Camp, 2012), modifying the relative strength of inhibitory or excitatory inputs (Turrigiano and Nelson, 2004), as well as adapting the thresholds for the expression of plasticity (Bienenstock et al., 1982; Bear et al., 1987; Bear, 1995; Figure 1-7B-C). The latter of these describes a mechanism wherein neighbouring synaptic weights are 'counterbalanced' following single input potentiation, such that when a single synapse increases in strength, neighbouring synapses decrease in compensation (Rabinowitch and Segev, 2006; 2008; Lee et al., 2013). Similarly, a global process that scales all synaptic weights up or down proportionally, regardless of previous potentiation, allows neurons to stabilize excitability and firing without changing the relative strength of synaptic inputs (Turrigiano, 2008; Turrigiano, 2012). These models allow for the stabilization of overall cell excitability by maintaining stable excitability within a dendritic compartment, branch, or whole cell.

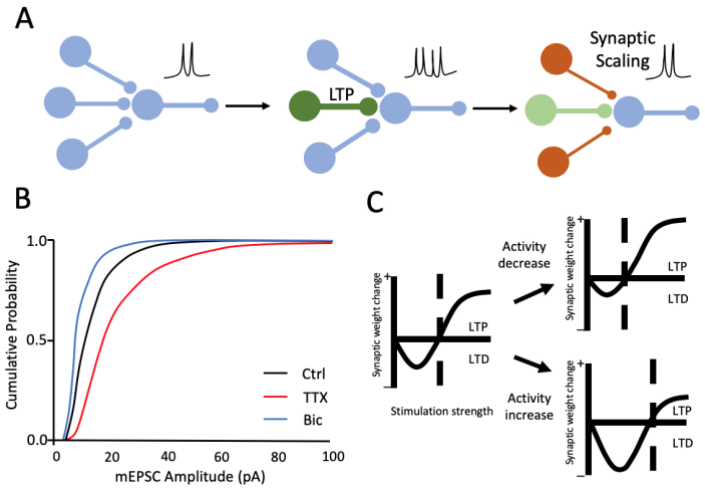


Figure 1-7 | Conceptual illustration of synaptic scaling. A) Schematic depiction of individual synapses (●). LTP of one input increases postsynaptic firing (●), homeostatic scaling will reduce the strength of both potentiated (●) and non-potentiated (●) synapses, to restore firing rate to baseline levels. B) Global increases (TTX) or decreases (Bic) shift the excitation of cultured neurons. C) sliding threshold for LTP and LTD induction. Increases or decreases in activity shift the threshold to promote LTD or LTP, respectively.

In the past decade, much effort has been made investigating mechanisms of homeostatic plasticity, first described by (Turrigiano et al., 1998). One method of homeostatic plasticity, synaptic scaling, has been investigated by measuring spontaneous miniature EPSPs (mEPSP) of neurons following treatment with GABA antagonists, such as bicuculline. This effect increases firing rates due to the prolonged decrease in network inhibition and the increase in spontaneous activity (Turrigiano et al., 1998). This group found that following treatment across a 48-hour period, mEPSPs returned to baseline values. Similarly, suppressing network excitability by overexpressing inward-rectifying potassium channels or application of the sodium channel blocker tetrodotoxin (TTX) was found to increase synaptic mEPSPs (Burrone et al., 2002; Gainey et al., 2009). Together these results indicate a

mechanism for compensatory, bi-directional control over cell excitability (Figure 1-7B). However, if Hebbian plasticity acts to increase or decrease the strength of individual inputs, and homeostatic plasticity acts to move a neuron back towards its original state, how does homeostatic plasticity avoid erasing the encoded Hebbian information? Early insights theorised that changes in neuronal excitability may shift the threshold for the induction of plasticity. From this, the Bienenstock, Cooper and Munro (BCM) learning rule was developed and describes a model in which decreases in activity result in a reduced threshold for LTP — promoting synapse strengthening and making LTD harder to achieve (Bienenstock et al., 1982). Conversely, increases in activity are proposed to shift the threshold to favour LTD and promote LTP (Figure 1-7C), and vice versa. The BCM rule has been shown to be applicable *in vivo,* such that light deprivation shifts the threshold for promoting LTP in the visual cortex as a means to promote changes in synaptic efficacy with minimal stimulation (Kirkwood et al., 1996). Many mechanisms have been thought to carry out homeostatic plasticity such as changes in the size of releasable presynaptic neurotransmitter vesicles (Murthy et al., 2001), release of neuromodulators from glial cells (Stellwagen and Malenka, 2006), as well as changes in the accumulation of glutamate receptors at the synapse (Turrigiano, 2008; Gainey et al., 2009; Pozo and Goda, 2010; Hou et al., 2011).

1.2.4. *Metaplasticity*

As has been made clear, synaptic plasticity is not a unitary event. Alterations in synaptic efficacy, including but not limited to LTP, LTD, and homeostatic plasticity, are made not only at the synaptic level, but at the level of the dendrite (Williams et al., 2007b), the cell (Debanne, 2009), the memory trace or engram (Bocchio et al., 2017), and network (Kajiwara et al., 2019). Importantly, these mechanisms do not occur in isolation, and mounting evidence suggests that previous synaptic and cellular activity alters the expression of subsequent plasticity at each of these levels. This plasticity of synaptic plasticity has been termed Metaplasticity, and is described as prior cellular or synaptic activity or inactivity which elicits a persistent change in the state of the synapse, altering the outcome of subsequent potentiation, depression, or expression of memory (Abraham, 2008). While metaplasticity appears most obvious when it occurs in the absence of altered synaptic efficacy, metaplastic modifications may also be induced by long-lasting changes, such as those occurring during LTP and LTD (Abraham and Bear, 1996). Early work showed that synaptic activity which, by itself, does not produce long-lasting changes, significantly influenced the degree of subsequent plasticity (Huang et al., 1992) such that the degree of LTP was significantly reduced. Further, both homeostatic plasticity (Kirkwood et al., 1996; Guo et al., 2012), and behavioural learning have been

shown to alter the induction of LTP (Tsvetkov et al., 2002; Whitlock et al., 2006), indicating that both non-hebbian and Hebbian-like mechanisms contribute to aspects of metaplasticity. Many of the mechanisms which have been found to influence metaplasticity involve the activation of NMDAR (Rebola et al., 2011), metabotropic glutamate receptors (mGluR; Gisabella et al., 2003), Ca^{2+} (Mahajan and Nadkarni, 2019), and protein phosphatases (Kato et al., 1999) and kinases (Gisabella et al., 2003; Young et al., 2006; Zorumski and Izumi, 2012). Importantly, metaplasticity has also been observed following application of neuromodulators, including dopamine (Otani et al., 2003; Sheynikhovich et al., 2013; Otani et al., 2015), nicotine (Huang et al., 2013), serotonin (Inoue et al., 2014), noradrenaline (Inoue et al., 2013; Keralapurath et al., 2014), BDNF (Rivera-Olvera et al., 2016), and tumour necrosis factor-α (TNF-α; Singh et al., 2019). In many cases these molecules act to prime synapses in order to enhance or suppress the induction of LTP, by shifting the threshold such that induction of LTP or LTD is more or less likely to occur.

1.3. Mechanisms Governing the Expression of Cellular Memory

So far, this review has aimed to summarise the characteristics which govern the expression of learning and memory at the cellular level. Early observations postulated that repetitive synaptic activity governs the metabolic change at the synaptic and cellular level. Research henceforth further characterised the role of high- or low-frequency stimulation in governing the synaptic potentiation and depression observed. Metabolic growth, initially postulated by (Hebb, 1949) has been further defined through the necessity of protein synthesis in regulating the persistence of memories, and gene expression in consolidating these changes. It is well understood that the genes and proteins expressed following the induction of LTP or LTD are strictly regulated, and govern discrete functions, each acting to directly or indirectly alter the postsynaptic potential. Henceforth, this review aims to address key questions which remain unanswered, firstly, what events occur during the initiation of LTP or LTD? Secondly, in what way are synapses altered following LTP or LTD, and how do these changes manifest into a persistent, stable memory? Thirdly, what is the role of growth factors in the induction and persistence of LTP? And lastly, how are these mechanisms altered in neurological diseases?

To answer these questions, I aim to characterise the relative contribution of proteins or molecules I believe to be innately linked to the expression of memory. Here, mediators, messengers, modifiers, and modulators form the foundations of a hierarchy, each contributing a specific set of functions in the expression of plasticity.

1.4. Mediators of Plasticity

Following the observations of the electrophysiological properties of LTP, LTD and homeostatic plasticity, research began to define the underlying molecular mechanisms which permit these changes. Much overlap exists between the proteins or processes responsible for many of the mechanisms which affect synaptic strength, however for the purpose of this literature review much emphasis will be placed on those which govern LTP. Consequently, I have defined four distinct categories of processes or molecule classes, which I believe to contribute to the expression of late LTP (LTP3) and plasticity in distinct ways. Mediators in this sense are defined as proteins or molecules, which are necessary for, and are directly involved in the mechanisms that lead to a change in synaptic strength. Genetic or chemical elimination of these mediators results in complete, or near complete obstruction of LTP, severely impairing plasticity, and ultimately affecting the expression of learning and memory.

1.4.1. *NMDA Receptors*

NMDA receptors (NMDAR) are ionotropic glutamate receptors with unique and extensive roles in mediating many of the changes during synaptic plasticity. NMDAR are heterotetrameric, formed from seven differentially expressed subunits: GluN1, which undergoes extensive splicing to produce eight variants (GluN1-1A–GluN1-4A & GluN1-1B–GluN1-4B), four GluN2 (GluN2A–GluN2D), and two GluN3 (GluN3A–GluN3B; Iacobucci and Popescu, 2017), produced from separate genes (*GRIN1-3*). Structurally, NMDAR are formed from the association of an obligatory GluN1 subunit with various GluN2 subunits to yield four major subtypes: GluN1/GluN2A, GluN1/GluN2B, GluN1/GluN2C, GluN1/GluN2D, characterised by distinct but overlapping regional and developmental expression patterns (VanDongen, 2008; Figure 1-8). Minor populations have also shown the association of GluN1 with GluN2A and GluN2B or GluN2C, as well as GluN1 with GluN2B and GluN2D, within the same receptor (Stephenson, 2001; Rauner and Köhr, 2011; Bhattacharya et al., 2018). Variations in GluN1 and GluN2A/2B compositions at the synapse regulate both the development of neural circuits, as well as the expression of plasticity (Sheng et al., 1994). The role of GluN3-containing subunits is not as clear; however, they appear to form functional receptors with GluN1, but not GluN2 subunits, primarily expressed during a short window of development (Pachernegg et al., 2012; Pérez-Otaño et al., 2016).

Following the characterization of NMDAR structure, many sought to determine the contribution of NMDAR to synaptic activity. It was Collingridge et al., (1983) who first described the sensitivity of hippocampal tissue to excitatory amino acids such as L-glutamate, and further described the dependence of NMDAR during LTP, finding that the NMDAR antagonist APV blocked LTP at Schaffer-collateral synapses. Around the same time, Lynch et al., (1983) described the importance of intracellular calcium (Ca^{2+}), by injection of the calcium chelator EGTA into the postsynaptic cell. This was found to significantly impair the induction of Schaffer collateral–CA1 LTP, indicating Ca^{2+} influx as a crucial mediator of downstream processes governing the expression of LTP in these cells. Importantly, later contributions found that NMDAR are highly permeable to both sodium (Na^{+}) and Ca^{2+} ions (Müller and Connor, 1991; Jahr and Stevens, 1993; Schneggenburger et al., 1993; McBain and Mayer, 1994; Garaschuk et al., 1996; Yu and Salter, 1998), and therefore may contribute significantly to processes of plasticity.

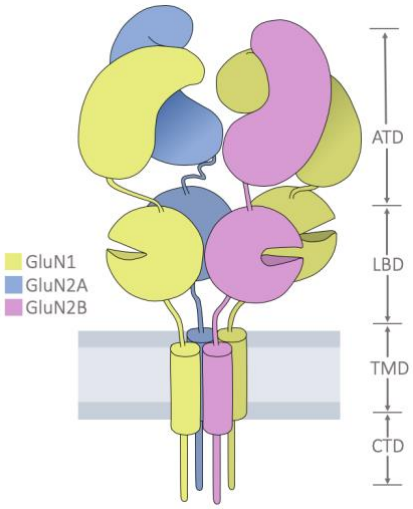


Figure 1-8| Schematic diagram of NMDAR Structure. Stylized diagram of GluN1 (■) and GluN2A (■) and GluN2B (■)-containing NMDAR showing amino terminal domains (ATD), ligand binding domains (LBD), transmembrane domains (TMD), and C-terminal domains (CTD).

From this evidence, it seemed reasonable to propose an NMDAR-dependent influx of postsynaptic Ca^{2+} as a mechanism through which synaptic changes could occur. Therefore, following these results, a series of papers were published which further delineated the details of this. In 1984, Nowak et al., showed the importance of a Mg^{2+}-dependent NMDA receptor blockade, such that in conditions in which extracellular Mg^{2+} was reduced, the NMDA receptor response was largely facilitated (Nowak et al., 1984). In 1985, Wigström and Gustafsson showed that blockade of postsynaptic inhibition by use of GABA inhibitors strongly facilitated LTP (Wigstrom and Gustafsson, 1985a), while Malinow and Miller showed that hyperpolarization of the postsynaptic membrane reversibly blocked LTP (Malinow and Miller, 1986). These properties suggest a 'membrane voltage-dependent' role of postsynaptic depolarization in regulating LTP. In this sense, NMDAR is thought to act as a coincident detector for activity sufficient to promote LTP and compute the Hebbian conditions necessary for associative memory. It has been proposed that ionic non-NMDAR channels (passing Na^{+} and K^{+}), or changes in the level of postsynaptic inhibition, is sufficient to provide postsynaptic depolarization and Mg^{2+} expulsion from the NMDAR channel, while consecutive presynaptic neurotransmitter release provides activation of NMDAR itself, promoting Ca^{2+} influx (Wigstrom and Gustafsson, 1985b). Later influential work by Morris et al., (1986) further tied NMDA

receptor activation directly to memory formation during a spatial learning task. Chronic intraventricular infusion of APV was found to selectively impair both the memory formation and the expression of LTP. More conclusive evidence from genetic ablation of CA1-specific NMDAR found a distinct absence of LTP at these synapses and an impairment in spatial memory (Tsien et al., 1996), suggesting NMDA receptors as an enticing mechanism of action in permitting the changes observed during long-term potentiation and memory.

1.4.2. *AMPA receptors*

In addition to NMDAR, many glutamatergic synapses house an abundant population of AMPA receptors. AMPA receptors comprise the primary excitatory receptor in the CNS, belonging to a class of ionotropic glutamate receptors further divided based on the individual subunit compositions, these include AMPA (GluA1–GluA4), kainate (GluK1–GluK5), delta (δ) receptors (GluD1 and GluD2), as well as NMDAR (Traynelis et al., 2010). AMPAR are composed of four subunits by a dimer of two identical homo- or heterodimers that together form a tetramer. Each subunit contains one ligand-binding site, and upon binding the subunit undergoes a conformational change allowing the channel to open and ions to flow through the pore (Mayer, 2005). The structure of each subunit can be divided into a large extracellular amino-terminal domain (ATD), ligand-binding domain (LBD), transmembrane domain (TMD) forming the pore, as well as a cytoplasmic carboxy-terminal domain (CTD, Figure 1-9). GluA1 and GluA4 (and a splice variant of GluA2, GluA2L) contain long carboxy-terminal domains, while these are short in GluA2 and GluA3 subunits (Diering and Huganir, 2018). These carboxy-terminal domains allow the subunits to interact with specific cytoplasmic and transmembrane proteins, due to the presence of phosphorylation, palmitoylation, ubiquitination, and nitrosylation sites along its length (Figure 1-9A).

The synthesis of AMPAR subunits and the subsequent assembly into functional receptors is a highly regulated process and occurs through two possible routes. The primary method occurs through the canonical synthesis and trafficking pathway (Hangen et al., 2018; Figure 1-10). This process begins in the soma, where AMPAR subunits are synthesised and assembled in the endoplasmic reticulum (ER), first forming dimers, followed by the formation of tetramers (Herguedas et al., 2013). Before release from the ER, AMPAR undergo a form of quality control through their associations to AMPAR-interacting proteins (Rubio and Wenthold, 1999; Hebert and Molinari, 2007), dependence on external signals such as Ca^{2+} (Lu et al., 2014; Pick et al., 2017; Hangen et al., 2018), and detection of conformational state (Penn et al., 2008), and edited RNA motifs (Greger et al., 2002). In the neuronal soma, AMPAR traffic from the ER to Golgi apparatus. Here, AMPAR may undergo extensive

posttranslational modifications (PTM), such as glycosylation, palmitoylation, and phosphorylation (Jiang et al., 2006). These PTMs are dynamic, reversible, and sensitive to alterations in synaptic activity (Jiang et al., 2006). From here, AMPAR may undergo transport from the soma towards target sites throughout the dendrites (Passafaro et al., 2001), or delivered directly to the somatic cell surface to undergo lateral diffusion (Borgdorff and Choquet, 2002; Adesnik et al., 2005). Transport throughout the dendrites occurs under the guidance of chaperone proteins such as kinesin and myosin motor proteins (Wang et al., 2008; Hoerndli et al., 2013), and the Ca^{2+}/calmodulin-dependent kinase (CaMKII; Hoerndli et al., 2015; Hangen et al., 2018), along microtubules into dendrites and

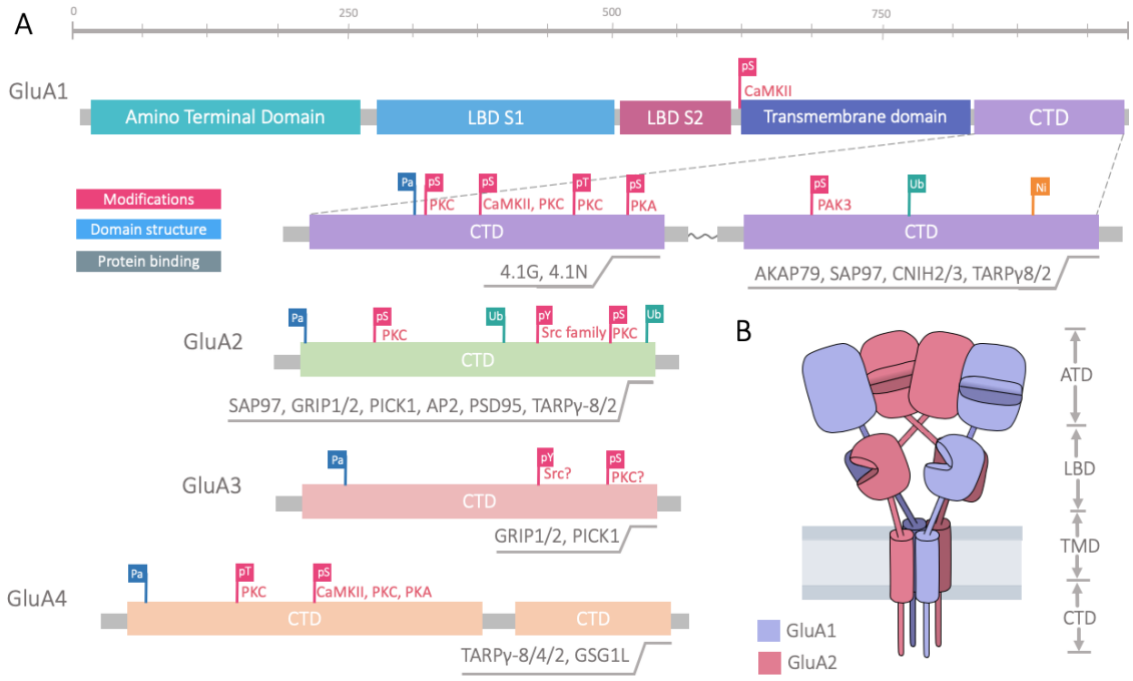


Figure 1-9 | Schematic diagram of AMPAR Structure. A) shows sites present in GluA1-4 subunits, including the amino terminal domain (ATD,), ligand binding domains S1 ( ) and S2 (LBD,), transmembrane domain (TMD,), and the C-terminal domain (CTD) of GluA1 ( ), GluA2 ( ), GluA3 ( ) and GluA4 ( ). Post-translational modifications include: phosphoserine (pS), phosphothreonine (pT), phosphotyrosine (pY), ubiquitinated lysine (Ub), nitrosylation (Ni). Scale bar: amino acids. B) Shows a stylized example diagram of the GluA1- ( ) and GluA2 ( )-containing heterodimers forming the tetrameric AMPAR structure.

spines.

Alternatively, AMPAR may also undergo synthesis at local sites within the dendrites (Torre and Steward, 1992; Kacharmina et al., 2000; Grooms et al., 2006; Sutton and Schuman, 2006). This method still involves the translation of mRNA by polyribosomes and ER located at the base of synapses (Steward and Levy, 1982; McCarthy and Milner, 2003; Ainsley et al., 2014), as well as specialized spine ER, called the spine apparatus (Frotscher and Deller, 2005; Jedlicka et al., 2008). However, as dendrites mostly lack the Golgi apparatus (Krijnse-Locker et al., 1995; Torre and Steward,

1996), AMPAR instead pass through a modified Golgi-related compartment, called the Golgi outpost (Horton and Ehlers, 2003; Horton et al., 2005). Conversely, recent evidence suggests AMPAR may undergo Golgi-independent trafficking, instead directly incorporating into the recycling endosome, following exit from the ER (Bowen et al., 2017; Figure 1-10). These mechanisms likely underlie distinct functional roles mediating the persistence of LTP (Vickers and Wyllie, 2007; Hangen et al., 2018).

1.4.2.1. *GluA1*

GluA1 (also referred to as GluR1 and GluRA) is undoubtedly the most studied AMPAR subunit in the context of synaptic plasticity and learning and memory. Considering its expression throughout many structures of the CNS, including the cortex, amygdala, cerebellum, thalamus, brainstem and the highest levels in the CA1–CA3 and DG regions of the hippocampus, it is clear to see why (Breese et al., 1996). GluA1 mRNA has been found extensively within both excitatory principal cells as well and inhibitory interneurons (Geiger et al., 1995), however expression has also been observed in GFAP-positive glial cells (Patneau et al., 1994; Matthias et al., 2003), as well as oligodendrocytes (Zonouzi et al., 2011; Evonuk et al., 2020). GluA1 has been shown to undergo both canonical *de novo* synthesis as well as activity-dependent local translation (Ju et al., 2004; Grooms et al., 2006; Cajigas et al., 2012), before highly regulated trafficking to the cell surface and synapse. GluA1 primarily forms heterodimers with GluA2 to form GluA1/2 AMPAR comprising approximately 80% of the total AMPAR population (Lu et al., 2009). However, it has also been shown to form GluA1 homomeric AMPAR (Lu et al., 2009) comprising 8% of the AMPAR population (Wenthold et al., 1996), yet the presence and contribution of there AMPAR is highly debated in the literature.

The differences in AMPAR subunit make-up impacts its trafficking and retention dynamics from the rough ER. While each subunit exhibits similar core structures, differences in the C-terminal domain (CTD) determine their trafficking dynamics in response to activity. Primarily, the long CTD present in the GluA1 subunit permits phosphorylation by activity-driven proteins such as Ca^{2+}/calmodulin kinase (CaMKII), and protein kinase A (PKA), PKC and PKG to regulate the trafficking and stabilization of GluA1-containing AMPAR (Hayashi et al., 2000), by facilitating associations with crucial chaperone and scaffold proteins such as PSD-95/Drosophilla discs large/zona occludens-1 (PDZ) domain proteins (Shi et al., 2001; Anggono and Huganir, 2012), including Synapse-associated protein 97 (SAP97; Leonard et al., 1998) A-kinase anchoring protein 79 (AKAP79; Colledge et al., 2000), and Cornichon Family AMPA Receptor Auxiliary Proteins 2/3 (CNIH2/3; Herring et al., 2013), transmembrane AMPA receptor regulatory proteins (TARPs) γ8 and γ2 (Diering and Huganir, 2018), and protein 4.1N (Shen et al., 2000).

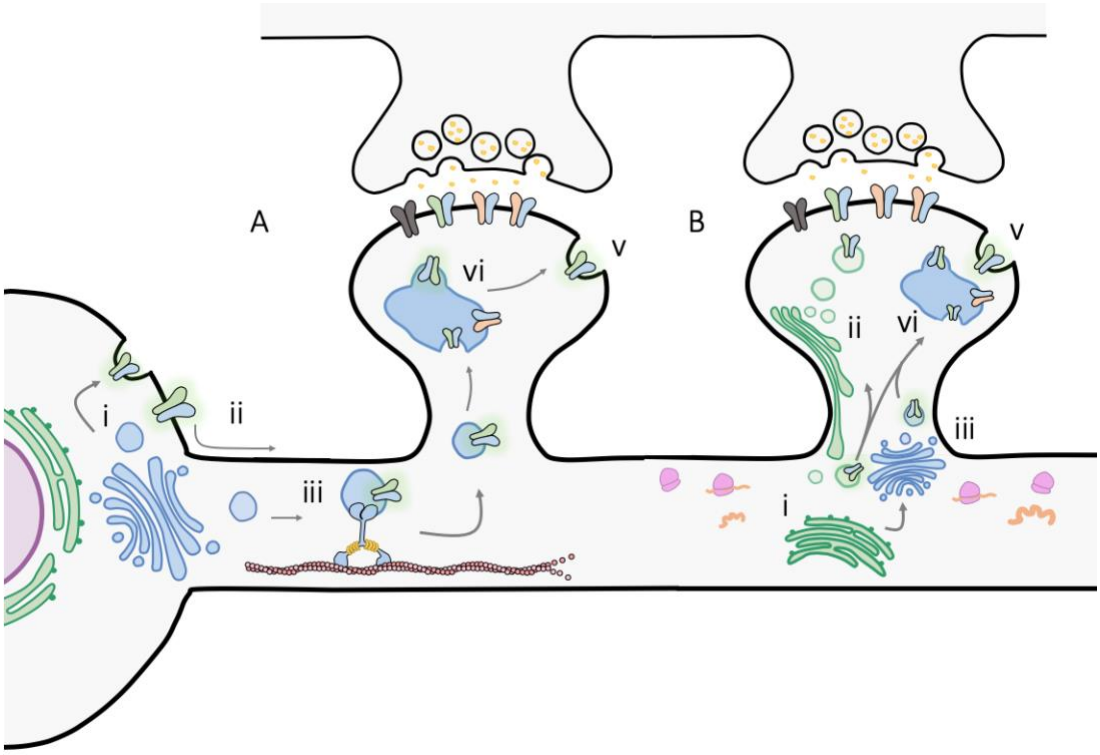


Figure 1-10 | Regulated AMPAR synthesis and trafficking methods. Schematic diagram shows both A) canonical and B) local mechanism governing AMPAR synthesis and trafficking. A) Following i) synthesis a within the soma AMPAR subunits exit the ER and pass though the Golgi apparatus, here AMPAR can be ii) exocytosed at the somatic cell surface and undergo lateral diffusion or iii) are trafficked within dendrites towards the synapse, where AMPAR are incorporated into vi) recycling endosomes before v) trafficking to the cell surface. Alternatively, B) AMPAR subunits may be synthesised locally at i) polyribosomes and ER or ii) spine apparatus present within the dendrites and spine. AMPAR may iii) pass through the Golgi apparatus or vi) incorporate directly into the recycling endosome before v) trafficking to the cell surface.

These associations permit GluA1 to contribute prominently to LTP through its activity-dependent regulation and delivery to potentiated synapses (Hayashi et al., 2000; Kakegawa et al., 2004; Williams et al., 2007a; Zhang et al., 2015; Yamasaki et al., 2016; Coombs et al., 2017; Bissen et al., 2019; Figure 1-9A). Conversely, inclusion of the shorter CTD present in GluA2 and GluA3 subunits results in longer retention within the ER, compared to AMPAR containing GluA1 subunits (Greger et al., 2003). Here, trafficking primarily occurs in a constitutive manner, thought to replace GluA1-containing AMPAR at the synapse (Araki et al., 2010; Casimiro et al., 2013). This trafficking is permitted through associations with PDZ protein such as N-ethylmaleimide sensitive factor (NSF; Nishimune et al., 1998), protein interacting with C-kinase 1 (PICK1; Lin and Huganir, 2007), and glutamate receptor-interacting protein 1 (GRIP1; Dong et al., 1997; Figure 1-9A). Within the CTD of GluA1 are six known phosphorylation sites: serine818 (Ser-818; Boehm et al., 2006) and threonine840 (t840; Lee et al., 2007) by protein kinase C (PKC), serine831 (Ser-831) by PKC and CaMKII (Barria et al., 1997a), serine567 (s567) by CaMKII, serine845 (s485) by PKA (Roche et al., 1996), and serine863

(s863) by P21-Activated Kinase 3 (PAK3; Park, 2018a; Figure 1-9A). Phosphorylation at these sites modifies the properties of the subunit by increasing channel conductance, increasing mean open probability, or permitting the trafficking of GluA1 to the cell surface or synapse (Hayashi et al., 2000; Lee et al., 2000). It has been generally accepted that phosphorylation of Ser-845 controls trafficking and retention of GluA1-contatining AMPAR, as well as enhancing channel open-probability to facilitate either LTP or homeostatic up-scaling (Banke et al., 2000; Esteban et al., 2003; Oh et al., 2006; Lee et al., 2010a), while dephosphorylation of Ser-845 has been shown to facilitate receptor endocytosis and LTD (Lee et al., 2010a). Conversely, phosphorylation of GluA1 at Ser-831 has been previously thought to enhance LTP through changes in single channel conductance (Derkach et al., 1999), which may now be understood as the trafficking of higher conductance Ca^{2+}-permeable AMPAR (Kim and Ziff, 2014; Yang et al., 2018; Park et al., 2019; Summers et al., 2019).

Following synthesis, trafficking of GluA1 to the cell membrane has been shown to be necessary for memory formation underlying fear conditioning (Rumpel et al., 2005), spatial memory (Sanderson et al., 2010), experience-dependent synaptic activity (Takahashi et al., 2003; Harms et al., 2005), and in both *in vitro* and *in vivo* LTP (Shi et al., 1999; Williams et al., 2007a; Zhang et al., 2015). While it is generally accepted that GluA1-containing AMPAR are delivered to the cell surface in an activity-dependent manner, the locus of exocytosis is greatly contested. Several studies have suggested that GluA1-containing AMPAR are initially inserted into the extrasynaptic dendritic membrane before laterally diffusing into the spine and PSD (Choquet and Triller, 2003; Adesnik et al., 2005; Ashby et al., 2006; Yang et al., 2008; Makino and Malinow, 2009; Figure 1-11) . However, it has also been suggested that AMPAR are inserted directly into both spines and perisynaptic sites lateral to the PSD (Yang et al., 2008; Kennedy et al., 2010). The dependence on these mechanisms has been deeply explored in a recent publication examining the role of AMPAR trafficking in LTP and memory formation (Penn et al., 2017). By cross-linking, and immobilizing cell surface GluA1/2-containing AMPAR, this group found that immobilization of surface pools of AMPAR

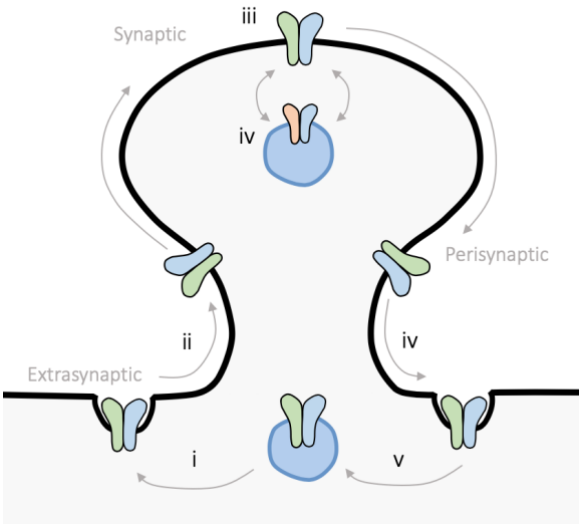


Figure 1-11 | Model of local AMPAR trafficking at the synapse. AMPARs enter the synapse by i) exocytosis at extrasynaptic sites, and ii) diffuse towards the perisynaptic domain and enter the PSD (iii). Here AMPAR may be exchanged directly by constitutive cycling (Gerges et al., 2006), or iv) diffuse out of the synapse to extrasynaptic sites to exit by endocytosis (v).

prevents both the induction and maintenance of LTP, as well as impairing the acquisition of contextual learning *in vivo*. Interestingly, permitting the mobility of surface receptors but restricting the diffusion of solely exocytosed AMPAR permits typical early induction of LTP, but impaired late phase consolidation. These results indicate that there may exist multiple steps in the regulation of synaptic AMPAR during LTP, governing distinct phases of potentiation.

Furthermore, a recent single-molecule trafficking study has sought to describe an alternate mechanism for AMPAR trafficking. (Morise et al., 2019) posit that AMPAR are unlikely to be formed as whole, functional tetrameric units within the ER, instead as individual monomeric AMPAR subunits which are trafficked to the cell surface, where they exist as monomers and dimers, rapidly (100-200 ms) exchanging in and out of tetrameric AMPAR assemblies, and able to diffuse rapidly in and out of the synapse, more readily than tetramers. Interestingly, GluA1/2 heterodimers and heterotetramers were found to be more stable than GluA1 homodimer and homotetramers. The formation of these complexes was found to be dependent on the interactions of N-terminus amino terminal domain (ATD) and C-terminal association with TARPγ2. Importantly, GluA1 homotetramers were capable of forming functional receptors, present at synapses and highly permeable to Ca^{2+} (Morise et al., 2019).

1.4.2.2. *Calcium-Permeable AMPA Receptors*

Calcium-permeable AMPA receptors (CP-AMPAR) are recognized as a rather elusive phenomenon within the memory and synaptic literature due to difficulties in detection (Shepherd, 2012), and their possibly transient nature, both developmentally (Gray et al., 2007; Ho et al., 2007; Lu et al., 2007; Sanderson et al., 2016), and following activity (Shi et al., 2001; Plant et al., 2006; Park et al., 2016; Morise et al., 2019). Regardless, CP-AMPAR have been detected throughout the CNS, including the hippocampus (Katsumaru et al., 1988; Koh et al., 1995), motor and auditory cortices (Otis et al., 1995; Angulo et al., 1999), amygdala (Mahanty and Sah, 1998), dorsal horn (Albuquerque et al., 1999), cochlear (Gardner et al., 1999; 2001), basal ganglia (Götz et al., 1997), and retina (Mørkve et al., 2002).

CP-AMPAR are formed through two possible mechanisms; firstly GluA1/2-containing AMPAR have been found to be permeable to Ca^{2+} under certain conditions. GluA2 subunit mRNA is subject to RNA editing by the ADAR2 enzyme (Horsch et al., 2011). ADAR2 converts the codon for glutamine (Gln607; Q) to arginine (Arg607; R). This editing restricts the flow of Ca^{2+} when in complexes with GluA1 and GluA3 subunits (Wright and Vissel, 2012), while unedited subunits permit Ca^{2+}influx. Interestingly, the presence of unedited GluA2 appears to accelerate GluA2 trafficking to the cell surface, while edited GluA2 AMPAR appear to show slower cycling rate, forming a more stable

recycling pool for later AMPAR formation (Greger et al., 2002). As GluA2 editing occurs in greater than 95–99% of all GluA2 mRNA (Isaac et al., 2007; Pachernegg et al., 2015), the contribution of unedited GluA2 in plasticity is unknown. Of note, a mouse line with genetically reduced Q/R editing showed a loss of hippocampal CA1 neurons, altered dendritic morphology and reductions in CA1 pyramidal cell spine density, as well as learning and memory impairments (Konen et al., 2020), indicating that chronic expression of GluA2-containing CP-AMPAR appears detrimental to neuronal health and plasticity. Alternatively, CP-AMPAR can be formed through the association of solely GluA1 subunits, forming homomeric GluA1 receptors (Rozov et al., 2012). The complete lack of GluA2 in this composition renders the receptor Ca^{2+}-permeable and inwardly rectifying, such that the outward ion flow is minimal, and at depolarised holding potentials (+40mV), CP-AMPAR exhibit a voltage-dependent block by endogenous polyamines. The rectification index (RI) is measured as the ratio of the AMPAR current observed between -60mV to that at +40mV, such that greater rectification displays a decrease in the RI. The presence of GluA2 is the determinant for this characteristic, if GluA2 is present, rectification of the AMPAR current does not occur at positive potentials, as Q/R editing renders the pore insensitive to polyamine blockade (Hayashi et al., 2000; Liu and Cull-Candy, 2000; Henley and Wilkinson, 2016). In addition to Ca^{2+}-permeability, CP-AMPAR are unique for displaying extremely fast kinetics and high conductance (Verdoorn et al., 1991; Geiger et al., 1995; Grosskreutz et al., 2003),

While GluA1 homomers are highly expressed during early periods of development (Pickard et al., 2000), in the adult CNS they appear absent from synapses, instead restricted to extrasynaptic or intracellular stores during basal conditions in adult animals (Lu et al., 2009; Yang et al., 2010; Jaafari et al., 2012). Yet, they have been found to contribute a significant proportion of AMPAR transmission following synaptic activity (Rozov et al., 1998) or the induction of LTP (Park et al., 2016; Plant et al., 2006; Guire et al., 2008; Lu et al., 2007; Yang et al., 2010; Yamanaka et al., 2017; Cepeda-Prado et al., 2019). However, these results have been contested by few groups, finding little to no contribution of GluA1 homomers to LTP (Adesnik et al., 2007; Gray et al., 2007). Regardless, genetic ablation of GluA1 in the hippocampus results in complete disruption of LTP at CA1 synapses (Zamanillo et al., 1999; Mack et al., 2001; Jensen et al., 2003; Selcher et al., 2012; Terashima et al., 2017), with animals experiencing disruptions in some (Reisel et al., 2002; Schmitt et al., 2004; Sanderson et al., 2007), but not all (Zamanillo et al., 1999) learning and memory processes.

Emerging bodies of evidence suggest CP-AMPAR play an important role in synaptic plasticity and memory formation (Man, 2011). Genetic knockout of GluA2 subunits in the mouse hippocampus has been found to enhance measures of both LTP and hippocampus-dependent learning tasks, due to

the increased likelihood that GluA1 subunits will associate to form homomers in the absence of GluA2 (Asrar et al., 2009; Wiltgen et al., 2010). A recent publication reported that spaced but not compressed theta burst stimulation (TBS) LTP protocols lead to the insertion of CP-AMPAR in a protein kinase A (PKA)-dependent manner (Park et al., 2016), while many others report similar findings in response to various LTP stimulation paradigms (Guire, et al., 2008; Lu et al., 2007 Yamanaka et al., 2017 Plant et al., 2006; Purkey et al., 2018), as well as experience-dependent plasticity *in vivo* (Clem and Barth, 2006; Williams et al., 2007a; Clem et al., 2008; Conrad et al., 2008; Clem and Huganir, 2010; Wen and Barth, 2012; Hong et al., 2013; Zhang et al., 2016; Ouyang et al., 2017; Suyama et al., 2017; Takemoto et al., 2017; Whitehead et al., 2017; Torquatto et al., 2019). However, some groups have provided evidence to suggest that CP-AMPAR do not govern all forms of LTP (Gray et al., 2007; Guire, et al., 2008; Adesnik et al., 2007; Purkey et al., 2018), and may be highly regulated by animal species and developmental age (Lu et al., 2007).

The trafficking and retention of CP-AMPAR at the synapse is under strict influence of activity-dependent phosphorylation. Phosphorylation of the Ser-831 site by CaMKII (Derkach et al., 1999) and PKC (Roche et al., 1996; Boehm et al., 2006) as well as Ser-818, also by PKC (Lin et al., 2009; Jenkins et al., 2014), regulates the trafficking of CP-AMPAR to the cell surface, while phosphorylation at Ser-845, a target of PKA phosphorylation, has been shown to be crucial for stabilization of CP-AMPAR at perisynaptic sites (He et al., 2009). Alternatively, dephosphorylation of Ser-845 during LTD removes perisynaptic AMPAR from the cell surface, underlying synaptic depression (He et al., 2009; Sanderson et al., 2012), yet insertion of CP-AMPAR may also underlie the induction of LTD, through low-frequency Ca^{2+} influx (Sanderson et al., 2016). Additionally, expression of the catalytic domain of the kinase CaMKI by viral vector in hippocampal neurons has been shown to promote the surface expression of CP-AMPAR (Guire et al., 2008), while the scaffold protein TARPγ2 has been shown to increase CP-AMPAR pore permeability and channel conductance through attenuation of the polyamine block (Panchenko et al., 1999; Soto et al., 2014).

Following accumulation at the PSD, CP-AMPAR are rapidly internalized and have been found to be quickly replaced by GluA2-containing AMPAR in an activity-dependent manner (Plant et al., 2006; Yang et al., 2010; Mattison et al., 2014). Preventing the interaction between GluA2 and trafficking proteins, or an absence of synaptic activity post-LTP induction prevents this switch, resulting in subsequent LTP mediated by CP-AMPAR. Further, knockout of GluA1 and GluA2 protein compromised initiation or completion of this synaptic exchange, respectively (McCormack et al., 2006), which may indicate distinct phasic roles for these subunits.

Importantly, the expression of CP-AMPAR at the synapse has been shown to promote a 'labile' synaptic state (Hong et al., 2013; Wright et al., 2020), such that activity governed through CP-AMPAR may underlie expansion and stabilization of the spine cytoskeleton during potentiation (Kopec et al., 2007; Fortin et al., 2010), while the synapse remains sensitive to depotentiation (Yang et al., 2008) until replaced by GluA2-containing Ca^{2+}-impermeable (CI-AMPAR; Wright et al., 2020). Through this, it is thought that CP-AMPAR mediate the induction of LTP by initially trafficking to perisynaptic regions and further localizing to the PSD. To account for the rapid delivery of GluA1-containing AMPAR to the PSD, is thought that the accumulation of these AMPAR initially occurs through lateral diffusion from extrasynaptic and dendritic membrane (Makino and Malinow, 2009; Petrini et al., 2009) and that later exocytosis of AMPAR acts to replenish these surface pools (Williams et al., 2007a). However, this finding has been contested as increases in GluA1 (but not GluA2) have been found at the synapse following *in vitro* LTP induction, followed by an increase in GluA2-containing AMPAR (Tanaka and Hirano, 2012), possibly supporting the notion for rapid GluA1 insertion and replacement of CP-AMPAR with GluA2-containing AMPAR.

While CP-AMPAR have been shown to be directly involved in the induction of many forms of LTP, CP-AMPAR may also contribute to supplementary mechanisms which underlie the formation of new synapses, through the unsilencing of silent synapses. Morita et al., (2013) have previously shown that immediately following the induction of LTP, previously AMPAR-silent synapses show complete or very strong inward rectification, indicating the incorporation of solely CP-AMPAR. A further 15 minutes of baseline stimulation results in a gradual decrease in rectification but the appearance of AMPA receptor-mediated EPSCs, indicating the insertion or replacement of CP-AMPAR with GluA2-containing AMPAR. Whether this indicates an 'and/or' mechanism of CP-AMPAR during LTP is unknown. However, the presence of CP-AMPAR has been further linked to the development of immature stubby, and thin spines following cocaine withdrawal-induced synapse unsilencing (Wright et al., 2020). This may indicate a strong role for CP-AMPAR in synapse formation, yet CP-AMPAR also appear to have a role in the growth and maturation of existing spines (Fortin et al., 2010).

Further, in addition to Hebbian plasticity, CP-AMPAR may also regulate many of the mechanisms regulating non-Hebbian homeostatic plasticity (Lissin et al., 1998; Thiagarajan et al., 2005; Goel et al., 2006; Aoto et al., 2008; Hou et al., 2008; Lee, 2012; Soares et al., 2013; Werner et al., 2017), an effect which has been observed by increases in solely surface GluA1 protein, or AMPAR rectification. However, there is some evidence that GluA2 is also involved in certain conditions (Wierenga et al., 2005; Anggono et al., 2011). Beyond neurons, homomeric CP-AMPAR play a pivotal role in regulating Ca^{2+} transients in glial cells including hippocampal astrocytes and Bergmann glial cells in the cerebellum (Enkvist et al., 1989; Muller et al., 1992; Jabs et al., 1994; Porter and McCarthy,

1995; Bezzi et al., 1998; Verkhratsky and Kirchhoff, 2007). Interestingly, CP-AMPAR have been shown to regulate neuron-glia LTP-like mechanisms (Ge et al., 2006), indicating a functional response to neurotransmitters released by neurons, mediated though CP-AMPAR. Together this research shows that the expression and regulation of CP-AMPAR in both a cell-type and activity-dependent manner may govern the expression of plasticity through a multitude of diverging mechanisms.

1.4.2.3. *GluA2, GluA3, and GluA4*

While GluA1 is referred to as the plasticity-promoting AMPAR subunit, GluA2- and GluA3-containing AMPAR appear to take place as AMPAR which consolidate these changes. GluA1/2 heteromers are abundant in principal cells of the hippocampus, comprising a majority of pyramidal cell AMPAR (Wenthold et al., 1996). Similar to GluA1 homomers, their trafficking is predominantly regulated by the presence of the GluA1 C-terminal, and is therefore considered to be activity regulated, participating in the plasticity-promoting induction of LTP (Isaac et al., 2007). The presence of the GluA2 C-terminal domain can permit exit from the ER through association with the PDZ domain protein, Protein Interacting With PRKCA 1 (PICK1) and CaMKII, however it may also regulate the retention of GluA2-containing AMPAR within endosomal compartments, permitting initial increases in GluA1-containing AMPAR at the cell surface, before release of GluA2-containing and replacement of CP-AMPAR (Jaafari et al., 2012). Due to the presence of the edited Arginine residue, GluA2-containing AMPAR are predominately non-rectifying, and solely permeable to sodium (Na^+) and potassium (K^+). Therefore, the addition of GluA1/2 AMPAR is thought to promote NMDAR-dependent plasticity by contributing to increasing the size of the postsynaptic potential. Importantly, knockout of the GluA2 gene (*Gria2*), results in enhanced LTP, fully governed by CP-AMPAR (Jia et al., 1996; Mainen et al., 1998; Meng et al., 2003). Interestingly, these animals express intact LTD, depotentiation, and basal synaptic transmission, until additional knockout of the GluA3 (*Gria3*) gene. Double *Gria2*$^{-/-}$*/Gria3*$^{-/-}$ knockout animals show significantly reduced basal synaptic transmission and impaired LTD and depotentiation, while LTP remains enhanced (Meng et al., 2003). Although LTP is enhanced GluA2 knockout animals, reduced performance on spatial working memory and spatial reference learning tasks have also been observed (Shimshek et al., 2006)

As mentioned above, the presence of the GluA1 C-terminal domain governs the activity-dependent trafficking of GluA1/2-containing AMPAR during plasticity. Replacement of the GluA1 CTD with the CTD of GluA2 has been shown to impair LTP, while replacement of GluA2 CTD with that of GluA1 has been shown to impair NMDAR-dependent LTD (Zhou et al., 2018). Using genetic overexpression of *Gria1* and *Gria2,* Shi et al., (2001) has further examined the contribution of GluA1-3

in activity-dependent and constitutive trafficking. Following the induction of LTP, homomeric GluA1-containing AMPAR were shown traffic primarily to previously silent synapses. Overexpression of GluA2, and formation of GluA2 homomers showed synaptic delivery in the absence of activity, occurring constitutively. Importantly, this shows that the presence of the CTD of GluA1- and GluA2 AMPAR subunits dictates the trafficking characteristics of GluA1- and GluA2- containing AMPAR, such that GluA1 homomers and GluA1/2 heteromers are under primary control of the activity-regulated GluA1 CTD. On the other hand, AMPAR containing the GluA2 CTD and lacking the GluA1 CTD, such as GluA2 homomers and endogenous GluA2/3 heteromers are likely governed by constitutive trafficking to replace synaptic AMPAR. However, as GluA2 homomeric AMPAR do not occur naturally, GluA1/2-containing AMPAR may in fact dictate an activity-regulated replacement of existing AMPAR. This has been described by (Liu and Cull-Candy, 2000) wherein activity at GluA2-lacking CP-AMPAR promotes the replacement of these AMPAR by non-rectifying GluA2-containing AMPAR.

Similar to GluA1, GluA2 can undergo extensive post-translation modifications through phosphorylation occurring at s863 and s880 by PKC and t876 by Src family tyrosine kinases (Henley and Wilkinson, 2013). The phosphorylation state of many of these sites dictates the binding affinity of associated auxiliary proteins including SAP97, Glutamate Receptor Interacting Protein 1/2 (GRIP1/2), PICK1, postsynaptic density protein 95 (PSD95), and TARPγ2 and γ8 (Henley, 2003; Czöndör and Thoumine, 2013; Hanley, 2014b; Herguedas et al., 2019). Interestingly, synaptic activity through CP-AMPAR has been shown to facilitate the replacement of CP-AMPAR by disrupting their interaction with GRIP1/2 and driving GluA2-containing AMPAR to the extrasynaptic membrane in a manner dependent on PICK1 (Liu and Cull-Candy, 2005), and lateral diffusion to the synapse in a manner dependent on NSF (Gardner et al., 2005). These results indicate that expression of GluA2-containing AMPAR is a dynamic process, involving both activity-dependent and constitutive mechanisms.

While the role of GluA1- and GluA2-containing AMPAR are well documented, the role of GluA3-containing AMPAR remains rather enigmatic. Next to GluA1/2, GluA2/3-containing AMPAR comprise the second most abundant heteromeric AMPAR in cortical principal cells, yet comprise only 10-30% of GluA1 and GluA2 levels (Geiger et al., 1995; Wenthold et al., 1996; Tsuzuki et al., 2001). Relative to GluA1-containing AMPAR, GluA3-containing AMPAR increase in expression throughout the hippocampus during development (Ritter et al., 2002). Likewise, in adult rats, approximately 80% of spines in the lateral amygdala are immunoreactive for GluA3, commonly following the same proportion as the NMDAR subunit GluN1 (Radley et al., 2007). Together, these allude to a role of GluA3-containing AMPAR in synapse maturity and stabilization.

GluA3-containing AMPAR do not contribute to canonical LTP, LTP-associated insertion of AMPARs, nor contextual fear memory (Meng et al., 2003; Humeau et al., 2007). However, in GluA1-deficent mice, β-adrenergic signalling has been shown to convert low-activity GluA3-containing AMPAR into plasticity-promoting receptors in CA1 neurons, resulting in GluA3-dependent plasticity (Renner et al., 2017). Regardless, during basal conditions, GluA3-containing AMPAR may act to stabilize changes in AMPAR receptor surface expression through slow constitutive recycling and replacement of synaptic GluA1/2-containing AMPAR (Passafaro et al., 2001; Shi et al., 2001; Malinow and Malenka, 2002). Similar to GluA2, GluA3 shares binding domains for PICK1 and GRIP1/2 (Xia et al., 1999; Silverman et al., 2007; Hanley, 2014), which regulate trafficking to the cell surface and stabilization within the synapse. In response to both LTD-dependent and constitutive endocytosis, interactions of the GluA2 CTD with the PDZ-domain protein PICK1 has been shown to be required for removal of GluA2/3-containing AMPAR from the cell surface (Kim et al., 2001). Mimicking GluA2 s880 phosphorylation prevents interactions with GRIP1/2 and excludes GluA2/3-containing AMPAR from synapses, depressing transmission, and partially occluding LTD. On the other hand, preventing s880 phosphorylation reduces the extent of LTD, indicating that s880 phosphorylation may disrupt GluA2 and GRIP1/2 interactions, facilitate removal of these AMPAR from the synapse (Seidenman et al., 2003), and promote their trafficking to and from recycling endosomes (Mao et al., 2010). Interestingly, both basal and LTD-dependent interactions with PICK1, and GRIP1/2 require the activity of PKC (Daw et al., 2000; Xia et al., 2000). While GluA1-containing AMPAR are thought to occupy the synapse by lateral diffusion from perisynaptic and extrasynaptic stores, some evidence suggests GluA3-containing AMPAR are inserted directly into the PSD (Gerges et al., 2006), supported by electron microscopy observations that GluA3-containing AMPAR predominately occupy the PSD, and lie more centrally within the PSD than GluA1-containing AMPAR (Radley et al., 2007; Jacob and Weinberg, 2015).

Converse to GluA3, GluA4-containing AMPAR show the greatest expression during early development (Ritter et al., 2002; Henley and Wilkinson, 2016), expressed primarily in the cerebellum, but are absent from the majority of excitatory neurons in the adult brain (Schwenk et al., 2014; Pelkey et al., 2015). The CTD of GluA4 contains a PKA/PKC phosphorylation site at s842 (Esteban et al., 2003). During early development, phosphorylation of s842 regulates activity-dependent recruitment and stabilization of GluA4-containing AMPAR at the synapse (Gomes et al., 2007), and may also be regulated by TARPγ2, γ4 and γ8 (Semenov et al., 2012; Pierce and Niu, 2019), as well as GSG1L (Keifer et al., 2017) auxiliary proteins. This period of development coincides with a period where PKA, but not CaMKII is required for hippocampal LTP (Yasuda et al., 2003), likely implicating a developmental

switch in activity-dependent AMPAR subunits. Alternatively, spontaneous synaptic activity has been shown to deliver GluA4-containing AMPAR to previously AMPA-silent synapses, allowing non-functional connections to be strengthened. These GluA4-containing AMPAR are later exchanged for GluA2 containing AMPAR in a manner requiring little neuronal activity (Zhu et al., 2000). However, following classical conditioning in freshwater pond turtles, GluA4 appears to show a predominant role in late phase consolidation of silent synapses (Mokin et al., 2007). Interestingly, parallels have been observed in the proportion of GluA4-containing AMPAR following spatial memory training in adult mice and classical conditioning in adult freshwater pond turtles, such that training increased both total and synaptic levels GluA4 (Keifer et al., 2008; Keihan Falsafi et al., 2012), while knockout of GluA4 impaired acquisition of spatial reference memory, with no effect on LTP or retention of spatial reference memory (Sagata et al., 2010).

1.4.3. *The Role of Calcium*

Following NMDAR activation, it has been accepted that the postsynaptic NMDAR-dependent influx of calcium initiates the essential signalling cascades required for the expression of NMDAR-dependent LTP. Synaptic potentiation has been observed following uncaging of a calcium-loaded calcium chelator Nitr-5 (Malenka et al., 1988), and alternatively, lowering the bath concentrations of calcium has been shown to selectively inhibit LTP (Dunwiddie and Lynch, 1979). Further, the kinetics of Ca^{2+} transients is tightly linked to the outcome of plasticity, such that differing outcomes of potentiation between cells can be predicted by the Ca^{2+} transients observed by each individually, thus the initial magnitude of postsynaptic Ca^{2+} increase is a critical variable in controlling the strength and duration of potentiation (Malenka, 1991b; Ismailov et al., 2004). Further, compartmentalization of Ca^{2+} transients have also been observed which may assist in the input specificity of LTP. Endogenous Ca^{2+} within dendritic spines is low, allowing for rapid changes in concentration, while diffusion across the spine neck is negligible, preventing the spread of Ca^{2+}-induced potentiation to neighbouring synapses (Sabatini et al., 2002). Crucially, Ca^{2+} gains its efficacy through the influence it has on downstream processes. Simply, Ca^{2+}, through its association with calmodulin, is the activity-dependent link between synaptic activity and downstream enzymatic activation (Eccles, 1983; Bliss and Collingridge, 1993; Suzuki, 1994; Malenka and Nicoll, 1999; Wu et al., 2006), and has therefore been defined as a key mediator in synaptic plasticity and potentiation.

1.4.4. *Calcium/Calmodulin-dependent Kinase II*

Following the discovery and identification of NMDAR and their role in plasticity, the field aimed to identify possible downstream targets of the NMDAR-dependent rise in intracellular Ca^{2+}. One of these targets was found to be the Ca^{2+}-dependent enzyme Ca^{2+}/calmodulin-dependent kinase II (CaMKII; Lisman et al., 2002). The major isoforms of the CaMKII gene consist of the α and β subunits which form dodecameric holoenymes consisting of either one or both subunit types. An appealing property of this molecule is its ability to autophosphorylate target sites within itself to remain active (site t286 on CaMKIIα subunits and t287 on CaMKIIβ subunits), even after intracellular Ca^{2+} has returned to baseline (Lisman, 1985). Mutation of this autophosphorylation site has been shown to not only prevent LTP but interfere with experience-dependent plasticity *in vivo* (Giese et al., 1998), while genetic CaMKII knockout mice showed deficiencies in the expression of LTP (Silva et al., 1992b) and impaired spatial memory (Silva et al., 1992a). Alternatively, introduction of active CaMKII into CA1 neurons potentiates the EPSC and occludes further LTP (Lledo et al., 1995). Thus, the activity of CaMKII has been proposed as one potential mechanism through which a cell or synapse may maintain a cellular correlate of 'memory-like' persistence (Lisman, 1994; Lisman et al., 2002). However, evidence shows that inhibition of CaMKII affects the induction of LTP and memory formation during contextual fear conditioning, but is not involved in maintenance of LTP, nor memory storage or retrieval *in vivo* (Buard et al., 2010). In line with this, stimulation of single dendritic spines has seen the activation and translocation of CaMKII to activated synapses within seconds following single synapse glutamate-uncaging or light-activation of channelrhodopsin-2 (Takao et al., 2005; Zhang et al., 2008; Lee et al., 2009), lasting for up to one hour post LTP-induction (Otmakhov et al., 2004). The rapid nature of CaMKII and its role in early LTP may be due to its abundant presence within the postsynaptic density (Kennedy et al., 1983; Petersen et al., 2003), forming complexes with GluN1, GluN2A, and GluN2B subunits of the NMDA receptor, regulating their association to synaptic scaffolding protein PDS-95 (Gardoni et al., 2006), as well as associating with other PSD proteins including densin-180 (Walikonis et al., 2001), synGAP (Oh et al., 2004), cytoskeletal proteins f-actin and α-actinin (Lin and Redmond, 2008; Jalan-Sakrikar et al., 2012), and the motor protein myosin Va (Costa et al., 1999), regulating trafficking to and from the synapse. The close association of CaMKII with these synaptic proteins and its localization within the PSD places CaMKII in prime position for incoming Ca^{2+} signalling (Gardoni et al., 1998; Leonard et al., 1999; Strack et al., 2000; Sanhueza et al., 2011), and a likely candidate for the regulation of synaptic processes during plasticity. Alternatively, CaMKII has also been show to translocate from within dendritic spines towards NMDAR-potentiated synapses during LTP (Thalhammer et al., 2006). This mechanism may add an additional amplification

to existing synaptic CaMKII, increasing the specificity of potentiated synapses, as well as mediate the potentiation and growth of naïve synapses (Strack et al., 1997; Rongo, 2002; Tsui and Malenka, 2006; Lisman et al., 2012).

A primary mechanism through which CaMKII potentiates LTP is through the trafficking and regulation of cell surface and synaptic AMPAR. CaMKII regulates phosphorylation of GluA1 AMPAR subunits at the Ser-831 and s567 sites (Barria et al., 1997a; Barria et al., 1997b; Mammen et al., 1997; Lu et al., 2010), of which Ser-831 is shared with PKC (Roche et al., 1996), while PKA regulates Ser-845 phosphorylation (Diering et al., 2016). Importantly, a bidirectional regulation of these phosphorylation sites has been described during LTP and LTD, such that during basal activity, cell surface GluA1-containing AMPAR are phosphorylated at the PKA Ser-845 site. Following LTP, these AMPAR are further phosphorylated by CaMKII at Ser-831, while LTD removes Ser-845 phosphorylation and permits endocytosis (Lee et al., 2000). Interestingly, CaMKII-mediated phosphorylation has been found to enhance AMPAR activity through two primary mechanisms; firstly by increasing single channel conductance of existing AMPAR following Ser-831 phosphorylation (Derkach et al., 1999), and secondly, by enhancing synaptic targeting of extrasynaptic GluA1-containing AMPAR by s567 (Lu et al., 2010). Interestingly, CaMKII may further regulate AMPAR expression indirectly by phosphorylation of the AMPAR auxiliary protein TARPγ2 (Hayashi et al., 2000; Opazo et al., 2010), promoting the trapping of extrasynaptic AMPAR within the synapse, likely through an increased association with PSD-95 (Ehrlich and Malinow, 2004b). The latter of these is thought to underlie a possible mechanism through which CaMKII may unsilence previously AMPAR-silent synapses (Poncer et al., 2002). Together, this evidence suggests both direct and indirect roles of CaMKII in promoting the induction of LTP (Diering and Huganir, 2018).

1.5. Messengers of Plasticity

Here, messengers of plasticity are defined as proteins or molecules which permit the action or regulation of proteins and processes which mediate plasticity. These proteins show distinct functions at the synapse, yet may comprise coinciding processes to regulate the induction, maintenance, and persistence of cellular memory. Many of the proteins described show clear evidence pertaining to the function, expression, and regulation of glutamate receptors, synaptic morphology, or related gene expression.

1.5.1. *The A–ζ of Protein Kinases*

Of the 250+ known protein kinases, only a few have been identified which contribute significantly to mechanisms of learning and memory. The idea that memory could be actively maintained by enzymatic activity was first established by Crick (1984), who proposed that persistently active enzymes could replace synaptic proteins necessary for maintaining changes that occur during learning (Crick, 1984). These kinases mediate changes in proteins by catalysing the addition of a phosphate group to specific protein substrates, which impact synaptic transmission through changes in ion channel expression, protein synthesis, and gene regulation (Giese and Mizuno, 2013). Of those shown to regulate synaptic plasticity, few have shown direct involvement with regards to the induction and maintenance of hippocampal LTP and LTD, through the regulation of glutamate receptors. Here, protein kinases A, C, and G will be discussed due to their direct roles in regulating AMPAR expression, protein synthesis, or immediate early gene (IEG) expression with relevant literature. Of note, PKB (also referred to as Akt), PKD, and PKN have also shown to play minor roles in the regulation of AMPAR expression (Pen et al., 2016; Zhao et al., 2019), and spine morphology (Dong et al., 2000; Taniguchi et al., 2001; Olayioye et al., 2013; Bencsik et al., 2015).

1.5.1.1. *Protein Kinase A*

Protein kinase A (PKA) is a tetrameric holoenzyme that consists of two catalytic and two regulatory subunits, initially identified in the 50s–60s (Sutherland and Rall, 1958; Walsh et al., 1968). Upon activation by Ca^{2+}, cyclic adenosine monophosphate (cAMP) binds PKA. The regulatory subunits dissociate from the catalytic subunits, and enable phosphorylation of target proteins. Interestingly, studies investigating mice with a genetic knockout of one of the catalytic subunits of PKA (Cα) shows

dramatic developmental impairments, significantly affecting growth and fertility in the few offspring which survive postnatally (Skålhegg et al., 2002). In contrast, knockout mice generated by ablation of the catalytic subunit $C\beta_1$ are phenotypically indistinguishable from wildtype littermates, with the only differences observed in the hippocampus, in which $C\beta_1$ knockout mice show impaired LTP, LTD, and depotentiation (Qi et al., 1996). These and additional findings have implicated PKA in both late phase LTP (Abel et al., 1997), and contextual fear conditioning (Bernabeu et al., 1997; Barad et al., 1998; Ahi et al., 2004; Isiegas et al., 2006). Interestingly, PKA expression may require protein synthesis, occurring between 1–6 hours post-training, placing its activity somewhere in the transition period between STP/E-LTP and L-LTP, and has been theorised to act in part as a 'synaptic tag' for capture of PRPs important for late phase LTP (Bernabeu et al., 1997; Bourtchouladze et al., 1998; Young et al., 2006).

Multiple targets of PKA phosphorylation have been identified, all of which play significant roles in mediating plasticity. Both NMDA and AMPA receptors have been shown to be tightly regulated by PKA activity (Greengard et al., 1991; Wang et al., 1991; Westphal et al., 1999; Skeberdis et al., 2006). PKA is associated with both NMDAR and AMPAR at the synapse through their affinity to PDZ-domain proteins PSD-95 and SAP97, and the formation of a complex with AKAP79/150 (Colledge et al., 2000). Importantly, another NMDAR binding protein, Yotiao, binds the calcineurin and protein phosphatase 1 (PP1) in close proximity to PKA (Westphal et al., 1999). Constitutive dephosphorylation of NMDAR by PP1 maintains low activity, whereas upon activation, PKA may phosphorylate PP1 to inhibit its activity, enhancing NMDAR currents and Ca^{2+} permeability (Michel and Scott, 2002; Skeberdis et al., 2006).

Further, PKA shows important roles in the regulation of cell surface GluA1-containing AMPAR, phosphorylating both Ser-831 and Ser-845 sites (Roche et al., 1996). Phosphorylation of Ser-845 is thought to enhance the trafficking of GluA1-containing AMPAR to the cell surface during both LTP (Ehlers, 2000; Esteban et al., 2003; Oh et al., 2006; Man et al., 2007), and synaptic upscaling during homeostatic plasticity (Diering et al., 2014). Importantly, opposing activity downregulates Ser-845 phosphorylated GluA1 from the cell surface, such that LTD or synaptic downscaling dephosphorylates GluA1 at Ser-845, removing GluA1-containing AMPAR from the synapse (Lee et al., 1998; Tavalin et al., 2002; Lee et al., 2003). Therefore, it is thought that maintenance of Ser-845 phosphorylation by PKA serves to maintain AMPAR at the synapse, likely through associations with AKAP79/150 and SAP97 (Diering et al., 2014). Interestingly, many of the associations between PKA and AKAP79/150 regulate the trafficking of CP-AMPAR during LTP, LTD or homeostatic plasticity (Kim and Ziff, 2014; Zheng and Keifer, 2014; Sanderson et al., 2016), primarily by interrupting the association of

AKAP79/150 associated calcineurin, which inhibits CP-AMPAR expression during basal activity (Sanderson et al., 2012; Purkey and Dell'Acqua, 2020). Of note, it has been proposed that both PKA and calcineurin exist in complex with AKAP79/150 and SAP97 on CP-AMPAR, such that NMDAR-dependent LTD promotes GluA2-containing AMPAR removal and simultaneous PKA-dependent CP-AMPAR insertion and translocation to the synapse. Low-frequency activity through synaptic CP-AMPAR is thought to promote activation of tethered calcineurin activity and rapid removal of CP-AMPAR from the synapse (Hell, 2016)

In addition to the role of PKA at the synapse, evidence suggests that PKA may play an additional role in regulating the activation of the cAMP response element-binding protein (CREB), a transcription factor with a crucial role in learning and memory (Impey et al., 1998; Athos et al., 2002; Abel and Nguyen, 2008; Kirschner et al., 2009). Importantly, the activation of CREB by PKA has been shown to regulate the expression of IEGs including *Arc*, *Zif268*, and *c-Fos* following synaptic activity (Didier et al., 2018), and LTP (Waltereit et al., 2001). In line with this, PKA has also been found to regulate the expression of long-term memories *in vivo* (Schafe et al., 1999; Schafe and LeDoux, 2000; Michel et al., 2011; Bollen et al., 2014). Therefore, the concerted regulation of both glutamate receptors and IEGs by PKA appears critical for the expression of many learning paradigms.

1.5.1.2. *Protein Kinase C*

In addition to PKA, protein kinase C (PKC) has been shown to regulate the expression of LTP and the formation of long-term memories. First isolated in the 80s, the PKC family consist of 10 isoenzymes, divided into three subgroups: conventional (α, βI, βII, and γ), novel (δ, ε, η, and θ) and atypical (λ and ζ; Coussens et al., 1986; Jaken, 1996), each of which are generally activated by Ca^{2+} and diacylglycerol (DAG). The first evidence that PKC could be involved in the mechanisms underlying memory comes from a publication in the mid-80s which found that application of phorbol esters, which bind to and activate PKC, or intracellular injection of PKC in rat hippocampal slices elicited a long-lasting enhancement of synaptic transmission, counteracted by PKC inhibitors (Hu et al., 1987; Reymann et al., 1988). Interestingly, inhibition of PKC has been found to impair the induction but not maintenance of LTP (Malinow et al., 1989; Muller et al., 1990). This data indicates that PKC may be required for early-phase potentiation, however some evidence contradicts this (Colley et al., 1990).

Importantly, similar to PKA, PKC has been shown to play a role in the regulation of cell surface GluA1-containing AMPAR. PKC shares the GluA1 Ser-831 phosphorylation site with CaMKII (Diering et al., 2016). As mentioned previously, the scaffold protein AKAP79/150 regulates the binding of PKC and calcineurin to GluA1 through associations with the synaptic protein SAP97. Additionally,

AKAP79/150 also binds PKC in this complex, and this association is said to promote preferential access of PKC over CaMKII to the Ser-831 substrate, following LTP (Tavalin, 2008). Through this, the AKAP79/150-PKA complex acts to regulate the phosphorylation and expression characteristics of GluA1-containing AMPAR. In the presence of AKAP79/150, PKC phosphorylation of Ser-831 promotes the preferential expression of CP-AMPAR at the synapse (Summers et al., 2019) and enhances single channel conductance (Jenkins and Traynelis, 2012). PKC has also been found to regulate the expression of GluA2-containing AMPAR, such that phosphorylation at the GluA2 PKC site s880 releases GluA2 from GRIP and enhances its interaction with PICK1 leading to increased internalization (Chung et al., 2000). This role has been found necessary for the expression of LTD (Matsuda et al., 2000; Kim et al., 2001), yet dedepression may also be dependent upon the activity of PKC, thought to occur by placing internal s880-phosphorylated GluA2/3-containing AMPAR into a constitutive pool for insertion (Daw et al., 2000).

A persistently active, brain-specific isoform of PKC (PKMζ), has been previously described as both necessary and sufficient for maintaining LTP. Application of the general PKC inhibitor chelerythrine chloride or the PKMζ-specific myristoylated zeta-pseudosubstrate inhibitory peptide (ZIP) to hippocampal slices significantly impairs potentiation following HFS (Ling et al., 2002), and erases consolidated spatial memories (Pastalkova et al., 2006). One of the mechanisms employed by PKMζ to enhance and maintain synaptic transmission is through its association with PICK1 and NSF. It is thought that PKMζ enhances trafficking of GluA2/3-containing AMPAR to the cell surface by enhancing NSF-GluA2 binding and disrupting interactions between PICK1-GluA2 (Yao et al., 2008; Migues et al., 2010). Interestingly, release of GluA2 from PICK1 via NSF has been shown necessary for the persistence of synaptic potentiation following CP-AMPAR-dependent LTP (Gardner et al., 2005), and therefore may describe a mechanism though which PKC (or PKMζ) maintains memory persistence following CP-AMPAR-mediated LTP induction.

The inhibition of conventional (Abeliovich et al., 1993; Bonini et al., 2007), novel (Hongpaisan et al., 2013), and atypical (Serrano et al., 2008; Ren et al., 2013; Schuette et al., 2016) isoforms of PKC impair the expression of LTP, as well as alter aspects of spatial and contextual conditioning. These data indicate that PKC plays a varied role in the regulation of LTP and memory.

1.5.1.3. *Protein Kinase G*

Protein kinase G (PKG) is a cyclic guanosine monophosphate (cGMP)-dependent enzyme (also referred to as cGMP-dependent kinase, or cGK) activated by soluble guanylate cyclase (sGC) following stimulation by nitric oxide (NO), guanylate cyclase (sGC; Giese and Mizuno, 2013). There exists two

PKG genes (*PRK1* and *PRK2*) encoding PKG type I (PKG-I, consisting of α and β isoforms) and type II (PKG-II), both of which are ubiquitously expressed, especially in the hippocampus, as well as the cerebellum, neuromuscular junction end plates, and kidney (Wu et al., 2018b). Conventional and conditional PKG knockout mice show impairments in cerebellar LTD and motor learning (Feil et al., 2003), as well as an age-dependent impairment in hippocampal protein synthesis-dependent LTP (Kleppisch et al., 2003). Further, direct or indirect inhibition of PKG has been shown to impair hippocampal CA1 LTP (Frey et al., 1993; Zhuo et al., 1994; Lu and Hawkins, 2002; Monfort et al., 2004). Interestingly, the role of PKG was initially thought to occur primarily through the regulation of presynaptic mechanisms, supported by observations that bath application, but not postsynaptic whole-cell filling of the cGMP inhibitor Rp-8-Br-cGMPS inhibits LTP (Blitzer et al., 1995) while the cGMP analogue 8-Br-cGMP enhances synaptic transmission (Arancio et al., 1995), in a manner independent of NMDAR activation (Son et al., 1998).

While much evidence suggests a presynaptic mechanism, evidence also exists for a postsynaptic mechanism. Importantly, NMDAR are directly linked to the activation of the NO-cGMP-PKG pathway. Early observations found that glutamate increased NO and cGMP levels in a manner dependent on NMDAR activation (Garthwaite, 1985; Chalimoniuk et al., 1996). These observations were later supported by the physical interaction between the NO precursor NO synthase (NOS) and GluN2B subunits of the NMDAR, bridged by PSD-95 (Brenman et al., 1996; Christopherson et al., 1999; Sattler et al., 1999; d'Anglemont de Tassigny et al., 2007; D'Mello et al., 2011). This close association allows for rapid activation of NOS by Ca^{2+} and calmodulin (Hayashi et al., 1999), and thus rapid downstream stimulation of sGC, cGMP, and PKG (Denninger and Marletta, 1999). Disruptions of this complex impair fear conditioned memory formation (Li et al., 2018), while activation of the NO-cGMP-PKG pathway has been shown to be important for fear memory consolidation (Ota et al., 2008), object recognition (Furini et al., 2010), and place preference (Shen et al., 2012; Shen et al., 2014), the latter of which was found to be dependent on the activation of GluN2B-containing NMDAR and the trafficking of GluA1-containing AMPAR (Shen et al., 2016).

Importantly, early investigations examined the necessity of NO as a retrograde messenger, such that postsynaptic activation of NMDAR promotes NO production and diffusion to the presynaptic cell (Schuman and Madison, 1991). However, key roles have also been identified within the postsynaptic cell primarily though PKG activation (Hardingham and Fox, 2006; Rameau et al., 2007), and enhancement of AMPAR expression at the synapse. Importantly, GluA1 AMPAR subunits contain PKG phosphorylation sites within the CTD, at the PKA Ser-845 and CaMKII/PKC Ser-831 site (Roche et al., 1996; Lu and Roche, 2012; Seo et al., 2013; Mao et al., 2014; Diering and Huganir, 2018). In line with this, recent evidence has shown that PKG colocalizes with and binds with the CTD of GluA1 *in*

vitro and *in vivo*, at a site distinct from the phosphorylation site (amino acids 850–873), following activation of cGMP cLTP (Serulle et al., 2007). This interaction enhanced the phosphorylation of GluA1 by PKG at Ser-845 and promotes the delivery of GluA1-containing AMPAR to the cell surface. Moreover, these actions occur independently of PKA, and unlike PKA does not require the binding of anchoring proteins AKAP79/150 and SAP97). In pathologically driven hyperexcitable states, such as temporal lobe epilepsy (TLE), PKG has been found to be upregulated, and inhibition of PKG *in vivo* prevents hyperexcitability though an inhibition of synaptic delivery of CP-AMPAR (Gu et al., 2018). These effects indicate that PKG may play a role in synaptic plasticity and LTP. In fact, application of nNOS, sGC, cGMP, or PKG inhibitors has been shown to significantly impair both the increase in mEPSC frequency following cLTP *in vitro* as well as the induction and persistence of LTP following TBS tetanisation *ex*- and *in-vivo* (4 pulses of 100 Hz at 5 Hz; Serulle et al., 2007). Inhibition of the PKG-GluA1 binding domain was found to be necessary for both the delivery of GluA1 to the cell surface following cLTP as well as the expression of LTP *in vivo*. Further, it has been shown that NO, cGMP and PKG promote the formation of new synapses and the trafficking of GluA1-containing AMPAR to the cell surface following cLTP (Wang et al., 2005). These findings have been further supported by the observation that enhancement of NO, cGMP or PKG increases the proportion of cell surface and synaptic CP-AMPAR, while silencing of PKG decreases CP-AMPAR expression (Incontro et al., 2013; Gu et al., 2018). Inversely, it has also been shown that activation of CP-AMPAR induces a PKG-dependent increase in GluA1-containing AMPAR, and a small increase in GluA2-containing AMPAR (Tukey and Ziff, 2013), possibly indicating that PKG may play a role in the receptor subunit switch following activity at CP-AMPAR (Liu and Cull-Candy, 2000; Plant et al., 2006). Further, NO activity may act as part of a downstream signalling cascade following CP-AMPAR activation, to regulate LTP (Haj-Dahmane et al., 2017)

In research investigating the behavioural effects of NO-cGMP-PKG signalling, it has been shown that NMDAR signalling in the lateral nucleus of the lateral amygdala (LA) following auditory fear conditioning drives the expression of IEGs Arc, c-Fos, and Zif268, while selective inhibition of this pathway inhibited fear memory consolidation and likewise impaired IEG expression (Ota et al., 2010), Similarly, inhibition of the NO-cGMP-PKG pathway *in vitro* attenuates the expression of c-Fos, Zif268, Arc, and BDNF following activity-induced homeostatic upscaling (Gallo and Iadecola, 2011).

Interestingly, reduced levels of cerebrospinal fluid (CSF) cGMP correlates with severity of dementia and depression in AD patients (Hesse et al., 2017), and inhibition of the cGMP-degrading enzyme phosphodiesterase (PDE5), has been shown to result in long lasting amelioration of synaptic and memory abnormalities in APP/PS1 mice (Zhang et al., 2013), as well as reductions in levodopa-

induced dyskinesia 6-OHDA-lesioned rats (Picconi et al., 2011). These properties have identified PKG as a key kinase in regulating mechanism of learning and memory, as well as a potential therapeutic target for many neurodegenerative diseases and neurological disorders including Huntington's disease (Saavedra et al., 2013) Parkinson's disease (Picconi et al., 2010) Alzheimer's disease (AD; Puzzo et al., 2009), and major depressive disorder (Reierson et al., 2011).

1.5.2. *Scaffold and Auxiliary Proteins*

More than one thousand proteins comprise the PSD, including neurotransmitter receptors, cell adhesion molecules, scaffold proteins, signalling enzymes, cytoskeleton proteins, and membrane trafficking proteins. The expression and synaptic localization of many of these proteins permit both basal transmission, as well as the full complement of synaptic plasticity. The major families of postsynaptic proteins are that of the membrane associate guanylate kinases (MAGUKs), which comprise PSD-93 and PSD-95 (Ehrlich and Malinow, 2004b; Ehrlich et al., 2007), and SAP97 and SAP102 (Elias and Nicoll, 2007), and that of the guanylate kinase-associated proteins (GKAPs) including GKAP, SAP90, PSD-95-associated protein 1 (SAPAP1), and discs large homologue-associated protein 1 (DLGAP1; Kim et al., 1997). Importantly, all of these scaffold proteins contain PDZ domains, capable of binding the CTD of NMDAR directly (Kornau et al., 1995; Niethammer et al., 1996), and indirectly to AMPAR through auxiliary proteins such as TARPγ2 (Schnell et al., 2002; Nicoll et al., 2006). This association may be enhanced by scaffold and chaperone proteins such as AKAP79/150, PICK1, GRIP1/2, NSF, and the AP2 adaptor complex which act to regulate the binding of downstream protein kinases and phosphatases during plasticity events (Dong et al., 1997; Colledge et al., 2000; Tavalin et al., 2002; Collingridge et al., 2004; Gardner et al., 2005; Smith et al., 2006; Jaafari et al., 2012; Nair et al., 2013). These findings have brought forward the 'slot' and 'scaffold' hypotheses of LTP and LTD (Xu, 2011). Such that PSD MAGUKs, in association with TARPγ2 (Bats et al., 2007), may mediate the maximum possible concentration of NMDAR and AMPAR at the synapse (Opazo et al., 2012). In fact, the formation of these 'slots' has been examined though the overexpression or knockout of PSD95 (El-Husseini et al., 2000), and to a lesser extent SAP102 (Schnell et al., 2002) and SAP97 (Schlüter et al., 2006). Overexpression of PSD-95 has been shown to selectively enhance basal synaptic transmission, mimic LTP (Ehrlich and Malinow, 2004), awaken previously silent synapses (Stein et al., 2003), and promote spine maturation (El-Husseini et al., 2000), while knockdown of MAGUKs reduces basal (Chen et al., 2015) and activity-driven (Ehrlich and Malinow, 2004) synaptic expression of both AMPAR and NMDAR, reduces *in vivo* LTP (Zhao et al., 2013), and impairs LTP-mediated spine morphology changes (Ehrlich et al., 2007). These observations indicate that scaffold

proteins are uniquely linked to the facilitation and regulation of synaptic plasticity through their associations to glutamate receptors and PSD proteins.

1.5.3. *Cytoskeletal Proteins*

A major aspect of cellular processes underlying plasticity includes the molecular and structural remodelling of the synapse, spine, dendrite, or major morphological changes to the entire cell (such as dendritogenesis and axonogenesis). In addition to changes in the postsynaptic potential following LTP, primarily governed through changes in the receptor content at the synapse, enhancements in potentiation are also shown to reflect changes in the postsynaptic morphology. Accordingly, plasticity-inducing stimulation has been shown to promote the formation of new dendritic spines (Parnavelas et al., 1973; Collin et al., 1997; McKinney et al., 1999; Wosiski-Kuhn and Stranahan, 2012), and the growth and stabilization of existing ones (Van Harreveld and Fifkova, 1975; Lee et al., 1980; Lendvai et al., 2000; Popov et al., 2004). Importantly, increases in spine size have been shown to be synapse specific (Jungenitz et al., 2018), correlates strongly with changes in AMPAR expression (Matsuzaki et al., 2001; Ganeshina et al., 2004; Matsuzaki et al., 2004), and are linked to *in vivo* behavioural learning, such as exposure to complex environments (Greenough and Volkmar, 1973), social isolation (Connor and Diamond, 1982), fear conditioning (Heinrichs et al., 2013; Xu et al., 2019), and working and spatial memory (Mahmmoud et al., 2015), indicating a strong correlation between memory formation and morphological changes to the synapse.

Many of these mechanisms involve the reorganization of the actin cytoskeleton (Lin et al., 2005; Korobova and Svitkina, 2010). Cellular actin exists in monomeric or globular (G-actin) and polymerized or filamentous (F-actin) forms. Importantly, F-actin remodelling and has been shown to regulate the trafficking, stabilization, and anchoring of AMPAR and NMDAR at the synapse (Allison et al., 1998; Mao et al., 2010), in addition to scaffolding proteins including PSD-95 (Pak et al., 2001; Mizui et al., 2005; Cingolani and Goda, 2008) and SAP97 (Waites et al., 2009; Hanley, 2014), while inhibition of F-actin stabilization has been shown to impair LTP (Kim and Lisman, 1999; Krucker et al., 2000). Seemingly counterintuitive, the Actin Depolymerizing Factor (ADF)/cofilin severs F-actin filaments, reducing the proportion of F-actin, but increasing the available pool of G-actin, creating actin nucleation sites and ultimately enhancing F-actin turnover. Inhibition of cofilin impairs AMPAR trafficking to the cell surface following LTP (Gu et al., 2010), reduces the surface motility of extrasynaptic AMPAR (Rust et al., 2010), restructures synaptic AMPAR nanodomains (Kerr and Blanpied, 2012), and results in impaired spatial memory performance (Rust et al., 2010).

An important aspect of AMPAR trafficking is the transport of AMPAR-containing vesicles across both large scale (soma to dendrite), as well as small scale (dendrite to synapse) distances. This is carried out primarily through associations between the F-actin cytoskeleton and myosin or kinesin motor proteins (Soldati and Schliwa, 2006). The best characterised of these are MyoV and MyoVI, which permit anterograde and retrograde directed movement, respectively (Hartman et al., 2011). MyoVI has been shown to associate with SAP97 and AP2 to promote the endocytosis of GluA1-containing AMPAR (Wu et al., 2002; Osterweil et al., 2005), while MyoVa, a subclass of MyoV proteins, can bind directly to the GluA1 C-terminus, and is necessary for the delivery of AMPAR to the cell surface during LTP (Correia et al., 2008). Conversely, MyoVb appears necessary for the delivery of Rab11-positive recycling endosomes into spines following LTP (Wang et al., 2008).

1.5.4. *Immediate Early Genes*

In addition to synaptic changes, mediated in part by interactions between glutamate receptors and spine scaffold and structure proteins, additional regulators of plasticity have been linked to strongly synaptic activity. These proteins include IEGs, defined as class of genes that show rapid upregulation in response to signals such as neurotransmitters and growth factors. Included in these are the transcription factors early growth response protein 1 (ERG-1, also known as Zif268), and the Jun (c-Jun, JunB, JunD) and Fos (c-fos, FosB, FosB2) families (Bahrami and Drabløs, 2016), as well as effector IEGs Homer (Vazdarjanova et al., 2002), Rheb (Yamagata et al., 1994), Narp (Tsui et al., 1996), β-A activin (Andreasson and Worley, 1995), and activity-regulated cytoskeletal-associated protein, Arc (Lanahan and Worley, 1998). While both transcription factor IEGs and effector IEGs are rapidly upregulated by synaptic activity, the contribution of transcription factor IEGs is indirect, involving the regulation of other genes, while effector IEGs appear to directly modify cellular function. Much of the literature surrounding IEGs has investigated their role in neuronal ensembles and memory traces. In the hippocampus, many spatial, and working memory paradigms induce strong transcription and expression of IEGs in select populations of task-specific neurons (Vann et al., 2000; Hall et al., 2001; Xie et al., 2014). Of these, Arc has been shown to elicit control of many aspects mentioned above, including F-actin reorganization, AMPAR trafficking, and gene transcription.

1.5.4.1. *Activity-Regulated Cytoskeletal-Associated Protein, Arc*

Originally discovered by (Lyford et al., 1995) and (Link et al., 1995) *Arc* mRNA was initially shown to be rapidly and specifically upregulated at activated synapses following high frequency stimulation (HFS; Wallace et al., 1998; Steward and Worley, 2001). The expression of *Arc* mRNA and the synthesis of Arc protein has been further shown to require Ca^{2+}, PKA, CaMKII, mitogen activated protein kinase (MAPK), eukaryotic elongation factor 2 kinase (eEF2K), and CREB signalling (Park et al., 2008; Kumar et al., 2012), driven by activation of ionotropic, metabotropic, and enzyme-linked receptors, including NMDAR (Bloomer et al., 2008), nicotinic acetylcholine receptors (α7nAchR; Kristensen et al., 2007), mGluR (Xia et al., 1996; Kumar et al., 2012), platelet-derived growth factor receptors (PDGFR), and tropomyosin receptor kinase B (TrkB; Yin et al., 2002; Kuipers et al., 2016).

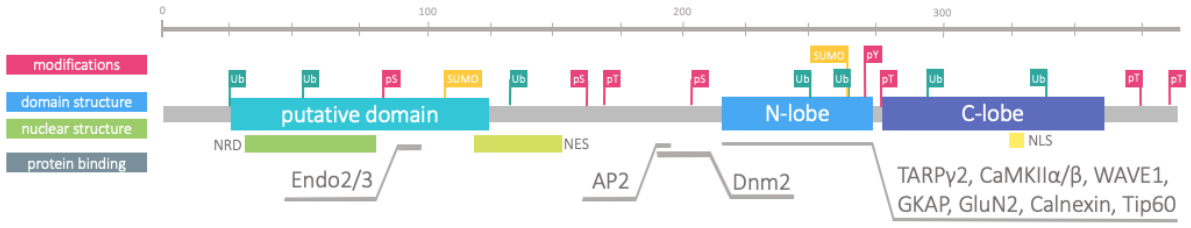


Figure 1-12 | Schematic diagram of Arc protein properties. Post-translational modifications include: phosphoserine (pS, ■), phosphothreonine (pT, ■), phosphotyrosine (PY, ■), ubiquitinated lysine (Ub, ■), SUMOylated lysine (SUMO, ■). Key sites include the nuclear retention domain (NRD, ■), nuclear export signal (NES, ■), and the nuclear localization signal (NLS, ■) within putative domain (■) and N- (■) and C-lobes (■). Dnm2 = dynamin-2, endo2/3 = endophilin-2/3. Scale bar = amino acids. Structure based of (Nikolaienko et al., 2018).

Following synthesis, Arc protein has been shown to associate with the cytoskeletal proteins F-actin, MAP2, Drebrin-A, and WASP-family verprolin-homologous protein (WAVE1), components of the clathrin-mediated endocytic mechanism, endophilin-3 (Endo3), dynamin-2 (Dmn2), and the clathrin adaptor complex AP2 (Fujimoto et al., 2004; Moga et al., 2004; Chowdhury et al., 2006; Rial Verde et al., 2006), the AMPAR auxiliary protein TARPγ2, the PSD proteins GKAP, α and β isoforms of CAMKII, and NMDAR subunits GluN2A and GluN2B, and Notch1 (Zhang et al., 2015), the histone acetyltransferase Tip60 (Wee et al., 2014), the endoplasmic reticulum protein, Calnexin (Myrum et al., 2017), as well presenilin-1 (PSEN1) as part of the γ-secretase complex (Figure 1-12). Through these associations, Arc has been linked to the regulation of cytoskeleton restructuring, gene expression, and the regulation and expression of both GluA1 (Chowdhury et al., 2006) and GluA2-containing (Rial Verde et al., 2006) AMPAR. This later function appears dependent on Arcs ability to interact with endophilin-3 and dynamin-2, indicating that Arc has the ability to actively promote the endocytosis of

GluA1 and GluA2-containing AMPARs from the cell membrane in an activity regulated manner. Through this, it is thought that Arc mediates the expression of LTP, LTD and homeostatic plasticity.

The induction of Arc synthesis upon synaptic activation, localization to active dendrites, association with key synaptic proteins, and regulation of cell surface AMPAR expression makes it a prime candidate for investigating the mechanisms underlying learning and memory. Due to this, previous publications have provided strong evidence for the necessity of Arc protein synthesis in the expression of LTP induction and consolidation. By negating the effects of Arc protein through conditional knockout (cKO; Plath et al., 2006) *in vivo* (in the DG) and *ex vivo* (in the CA1), or infusion of antisense oligodeoxynucleotides (ODNs; Guzowski et al., 2000; Messaoudi et al., 2007) *in vivo* (DG), these groups provided initial evidence that both persistent and acute blockade of Arc function alters the expression of LTP. Primarily, all groups found a significant deficit in the persistence of late phase LTP, lasting between 90 minutes to 5 days following acute (Messaoudi et al., 2007), and persistent (Guzowski et al., 2000) ODN treatment, respectively. In both cKO and persistent ODN experiments, blockade of Arc protein expression impaired spatial memory (Guzowski et al., 2000; Plath et al., 2006), as well as context fear conditioning, conditioned taste aversion, and object recognition in cKO mice (Plath et al., 2006). Interestingly, this work has been recently examined through a comprehensive approach of Arc KO and cKO mice (Kyrke-Smith et al., 2020), utilizing both HFS and TBS LTP induction protocols. Here, the authors describe a lack of dependence of Arc expression on the persistence of LTP in both KO and cKO mice using both HFS and TBS induction protocols. The authors note differences in anaesthetic regime and Arc knockout strategy, which may account for differences in the expression of LTP.

Importantly, in Arc cKO (Plath et al., 2006) and KO (Kyrke-Smith et al., 2020) mice, but not ODN infused rats, the initial induction of LTP was significantly enhanced, exceeding that of wild-type (WT) mice by 50% (Plath et al., 2006), before rapidly returning to baseline by 90 minutes in the case of (Plath et al., 2006). This may reflect differential regulation of basal levels of cell surface AMPAR following persistent and acute blockade of Arc. Such that, the delivery of AMPAR following synaptic activity in cKO/KO mice may result in the accumulation of AMPAR at the extrasynaptic membrane, priming the induction of LTP. Acute blockade of Arc by ODN 5-90 minutes before the induction of LTP minutes (Guzowski et al., 2000; Messaoudi et al., 2007) may not be sufficient to expand these extrasynaptic pools (Kennedy and Ehlers, 2011).

Importantly, the observed differences in the necessity of Arc protein in the regulation of late-phase LTP, may also be due to the expression and requirement of CP-AMPAR. Due to due to Arc's role in receptor endocytosis it is thought that the increase in potentiation observed by both Plath et al., (2006) and Kyrke-Smith et al., (2020) may be due to increased expression and availability of

extrasynaptic CP-AMPAR. Overexpression of Arc has been shown to decrease cell surface expression of CP-AMPAR (Lanté et al., 2011; DaSilva et al., 2016; Scheyer et al., 2018), and may permit the insertion of CI-AMPAR (Wall et al., 2018). If the induction protocol used by Plath et al., (2006; *in vivo:* 6 series of 6 trains of 6 stimuli at 400 Hz, 200 ms, *ex vivo*: 200 pulses at 1.5 Hz + postsynaptic depolarization) is sufficient to induce the induction of CP-AMPAR in these animals, but not the induction protocol used by Kyrke-Smith et al., 2020; 4 trains of 10 bursts at 5Hz containing 4 stimuli at 100Hz), LTP in absence of Arc may saturate synaptic AMPAR and impair the removal of CP-AMPAR, preventing the exchange for GluA2-containing AMPAR and impairing the persistence of LTP (Liu and Cull-Candy, 2000). Alternatively, it is possible the induction protocol used by (Kyrke-Smith et al., 2020) induced CP-AMPAR dependent LTP, and the increase in both induction and persistence *ex vivo* and increased induction *in vivo* may result from LTP governed solely by CP-AMPAR, due to impaired endocytosis of CP-AMPAR.

In addition to mediating aspects of LTP, Arc also plays a role in the regulation of LTD. Arc cKO mice show reductions in the extent of both the induction and persistence of LTD *in vivo* (Plath et al., 2006), acute siRNA-mediated blockade of Arc prevents the maintenance, but not induction of LTD (Park et al., 2008; Waung et al., 2008). This mechanism appears more directly linked to impairments in the endocytosis of both GluA1- and GluA2/3-containing AMPAR as overexpression of Arc occludes LTD through a mechanism requiring the removal of GluA2/3-containing AMPAR (Rial Verde et al., 2006), while knockdown of Arc protein impairs mGluR-mediated endocytosis of GluA1- and GluR2/3-containing AMPAR. Interestingly, while NMDAR may be required for the induction of Arc following LTP (Bloomer et al., 2008; Yilmaz-Rastoder et al., 2011), NMDAR-dependent LTD appears to function independent of Arc function (Rial Verde et al., 2006), and in fact may negatively regulate *Arc* mRNA (Yilmaz-Rastoder et al., 2011). It is thought that past activity, such as novel environment exposure, induces dendritic transport of *Arc* mRNA and primes neurons for local synthesis of Arc protein during mGluR-mediated LTD (Jakkamsetti et al., 2013). Importantly, Steward et al., (2015) posit that if increased Arc activity is linked to the endocytosis of AMPAR, strong Arc expression following high periods of synaptic activity, such as electroconvulsive stimulation (ECS; Lyford et al., 1995; Steward and Worley, 2001) should result in a rundown of synaptic efficacy *in vivo* during the period of high Arc expression. Interestingly, this group found that following ECS, strong activation of Arc expression did not result in a depression of synaptic responses (Steward et al., 2015). This work indicates that Arc may play a more complex role in the maintenance of synaptic transmission.

Lastly, Arc has also been implicated in the mechanisms of homeostatic scaling through its removal of GluA1- and GluA2-containing AMPARs from the synapse and cell surface. In primary hippocampal cell

cultures, experimentally induced inactivity with the sodium channel blocker tetrodotoxin (TTX), or increased activity with the $GABA_A$ antagonist bicuculline has been shown to result in increased and decreased surface GluA1 AMPAR expression, respectively. Overexpression or knockout of Arc protein occludes these homeostatic scaling responses, such that overexpression of Arc decreases basal expression of GluA1 AMPARs and impairs synaptic downscaling, while knockout of Arc increases basal cell surface GluA1, preventing the upscaling of AMPAR following activity blockade (Shepherd et al., 2006). Conversely, Arc has also been found to selectively reduce synaptic GluR2/3 AMPARs (Rial Verde et al., 2006). Here, reductions in AMPAR transmission, following overexpression of Arc in organotypic hippocampal slices, were occluded by expression of the GluR2 C-terminal tail, preventing removal from the cell surface. Biotinylation of cell surface proteins saw a reduction in GluA2, with no effect on surface GluA1.

Following these findings, authors note that the disparities between outcomes observed by Shepherd et al., (2006) and their own observations, and posit that differences in the recycling dynamics of GluA1- and GluA2-containing AMPAR in cell cultures versus organotypic slices may be responsible. Slow recycling of GluA1-containing compared to GluA2/3-containing AMPAR in hippocampal slice cultures may explain the selectivity for GluA2/3 over GluA1-containing AMPAR, while GluR1-containing AMPAR recycling is much faster in primary cultured neurons. Further, cultured neurons obtained from young animals may differ in the expression of subunit composition compared to tissue obtained from adult animals. The expression of GluA1-containing CP-AMPAR is higher in tissue obtained from young animals than that in adult tissue (Takemoto et al., 2017). Therefore, internalization of AMPAR may arise from the expression or availability of AMPAR for endocytosis, rather than selectivity governed by Arc itself, and thus conclusions from overexpression and knockout studies must be interpreted from the perspective of the system in which it is examined.

Additionally, Arc has also been found to be necessary for synapse-specific homeostatic scaling, by mediating the synaptic upscaling of GluA1-containing AMPARs (Béïque et al., 2011). Here, chronic inactivation of glutamate release from the single presynaptic terminals showed a homeostatic increase in CP-AMPAR. However, within Arc-KO cells, these synapses fail to promote this upregulation in synaptic AMPARs. It is thought that this may occur through an occlusion-like mechanism, such that Arc may be required for enhancing the trafficking of GluA1 to the synapse. Alternatively, persistent Arc expression may be required for the formation and maintenance of internal AMPAR reserve pools (Béïque et al., 2011).

Regardless, following the observed interactions of Arc with AMPAR regulatory proteins and Arc's proposed regulation of both GluA1- and GluA2-containing AMPAR, the following mechanism has been proposed (Hanley, 2018; Wall and Corrêa, 2018; Figure 1-13);

1) GluA1- and GluA2-containing AMPAR at the synapse are bound to PSD-95 via TARPγ2. Following LTD stimulation, dephosphorylation of TARPγ2 disrupts the association with PSD-95 while Ca^{2+} enhances GluA2-PICK1 binding (Citri et al., 2010). Alternatively, following LTP, phosphorylation of TARPγ2 enhances PSD-95 binding (Tomita et al., 2005).
2) Arc protein associates with the AP2 complex, which binds GluA1 via PDS-95-bound TARPγ2 (Matsuda et al., 2013), or GluA2 via PICK1, at the plasma membrane (Lee et al., 2002; Biou et al., 2008; Garafalo et al., 2015; DaSilva et al., 2016). This selectivity may define the differential recruitment of activity driven GluA1-containing and CP-AMPAR, and more stable GluA2/3-containing AMPAR populations.
3) Calcineurin enhances AP2-PICK1 binding, and AP2 recruits clathrin to the membrane to initiate the formation of the clathrin-coated pit (CCP) assembly. AP2-PICK1 interaction disrupts GluA2-PICK1. Formation of the CCP decreases the binding affinity of AP2 and Arc and this complex dissociates (Kelly et al., 2014).
4) Dissociation of Arc from AP2 frees the same binding motif used by dynamin-2 as well as binding endophilin. The binding of dynamin-2 and endophilin-3 promotes the scission of the endocytic vesicle containing the AMPAR (Sundborger et al., 2011).

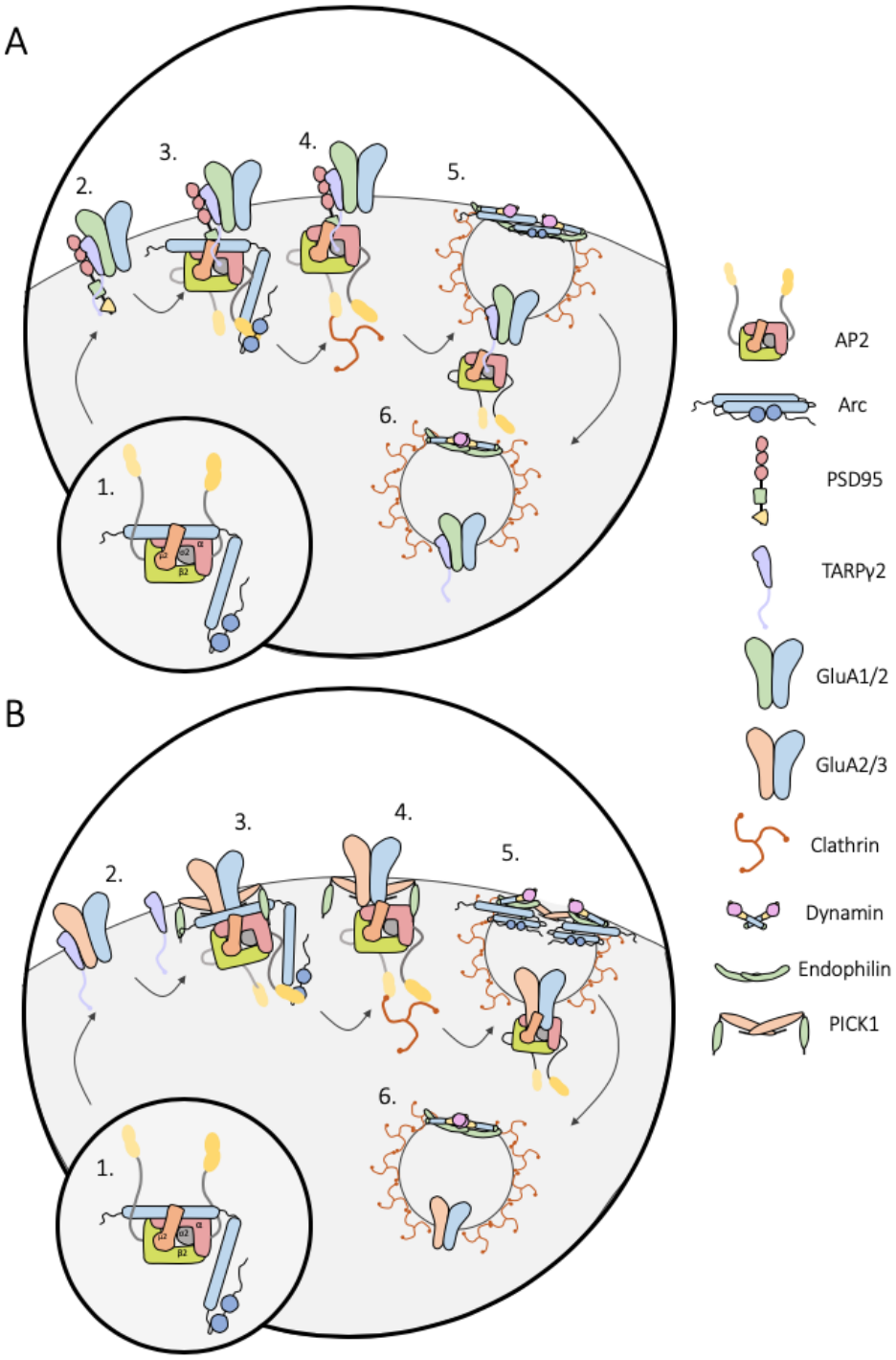


Figure 1-13 | Regulation of GluA1 and GluA2 endocytosis by Arc. Arc has been hypothesised to differentially regulate cell surface GluA1 and GluA2-containing AMPAR expression through proposed protein-protein interactions.

A) Arc regulates GluA1-containing AMPAR. 1) Following activity, Arc binds AP2. 2) GluA1 binds PSD-95 via TARPγ2. 3) AP2 binds GluA1 via TARPγ2. AP2 recruits clathrin and initiates formation of the clathrin-coated pit (CCP). 5) Arc dissociates and binds dynamin and endophilin to enable scission of the CCP from the membrane. 6) GluA1-bound vesicle is then internalized.

B) Arc regulates GluA2-containing AMPAR. 1) Following activity, Arc binds AP2. 2) TARPγ2 dissociates from GluA2, PICK1 binds GluA2. 3) Arc-AP2 binds PICK1. 4) Arc dissociates from AP2, AP2 recruits clathrin. 5) Arc binds dynamin and endophilin to enable scission of the CCP from the membrane. 6) GluA2-containing vesicles are internalized.

In addition to regulating synaptic AMPAR, Arc expression may also play an important role in the dynamic regulation of morphological plasticity (Figure 1-14). As previously shown by Messaoudi et al., (2007), infusion of Arc antisense ODNs into the rat dentate gyrus 2 hours post-HFS impaired the persistence of LTP. This effect was found to be dependent on Arc-mediated phosphorylation of cofilin. Dephosphorylation of cofilin allows for nucleation and polymerization of F-actin filaments by Arp2/3 (Smith et al., 2013), promoting trafficking of AMPAR to the cell surface (Wang et al., 2013), while phosphorylation of cofilin inhibits F-actin polymerization, consolidating spine growth, and stabilizing synaptic AMPAR, following activity (Fukazawa et al., 2003; Chen et al., 2007; Gu et al., 2010; Calabrese et al., 2014). Interestingly, within one hour following HFS, Arc has been previously found to undergo SUMOylation, a post-translational modification involving the addition of a small ubiquitin-like modifier (Nair et al., 2017). This SUMOylation promotes the association of a complex with the F-actin binding protein Drebrin-A in synaptoneurosomal and cytoskeletal fractions *in vivo.* Debrin-A has been previously shown to be important in regulating actin stabilization (Hayashi and Shirao, 1999; Kojima and Shirao, 2007; Ivanov et al., 2009; Mikati et al., 2013) by promoting phosphorylation and inhibition of cofilin activity (Grintsevich and Reisler, 2014). The binding of SUMOylated Arc protein to Drebrin-A is thought to promote the dephosphorylation of cofilin and polymerization of F-actin. Therefore, Arc may regulate actin dynamics in a two-step manner, through both SUMOylated and non-SUMOylated functions. Firstly, within one hour, a fraction of the total pool of newly synthesised Arc undergoes SUMOylation, associates with Drebrin-A and exits the spine head (Koganezawa et al., 2017), permitting dephosphorylation and activation of cofilin. In turn, cofilin promotes F-actin polymerization, growth of the actin cytoskeleton, trafficking of AMPAR to the cell surface, and induction of LTP. Secondly, within 2 hours, the return of Drebrin-A to the spine promotes phosphorylation and deactivation of cofilin, stabilizing the F-actin cytoskeleton and anchoring synaptic AMPAR. Supporting this idea is the observation that SUMOylated Arc has been found to be required for the upscaling of AMPAR following inactivity (Craig et al., 2012), possibly by promoting the trafficking of AMPAR to the cell surface (Henley and Wilkinson, 2013; Figure 1-14).

Interestingly, SUMOylated Arc does not coprecipitate with CaMKIIα, CaMKIIβ, PSD-95 and dynamin-2, unlike non-modified Arc (Nair et al., 2017). Therefore, it is possible this modification allows for distinction between separate mechanisms such that spine expansion during early phase LTP, may occur concurrently or independently to Arc's endocytosis of AMPARs and spine stabilization following periods of high activity (Craig et al., 2012; Hanley, 2014). Furthermore, Huang et al., (2007) have shown that following HFS, local inhibition of actin polymerization by infusion of a Rho kinase inhibitor blocked *Arc* mRNA localization to the activated dendritic laminar. These results likely indicate a positive feedback, wherein locally translated Arc is initially SUMOylated, associating with Drebrin-A

and enhancing F-actin polymerization. This in turn may allow localization of *Arc* mRNA to activated synapses, where non-modified Arc binds CaMKIIα, CaMKIIβ, PSD-95 and dynamin-2 to regulate consolidation of LTP and LTD.

Interestingly, Arc may play an additional role in the trafficking of GluA4-containing AMPAR to the synapse during classical conditioning (CC), in freshwater pond turtles (*Pseudemys scripta elegans*). Here, CC has been found to increase GluA4-containing AMPAR at the synapse (Mokin and Keifer, 2004), as well as enhancing the rapid colocalization and co-immunoprecipitation of Arc with both actin and GluA4-containing AMPAR (Mokin et al., 2006). This data is thought to described a role for Arc in the synaptic targeting of GluA4-containing AMPAR during CC (Mokin et al., 2007), through an association with the actin cytoskeleton and the selective localization of GluA4-containing AMPAR to previously silent synapses (Mokin et al., 2007).

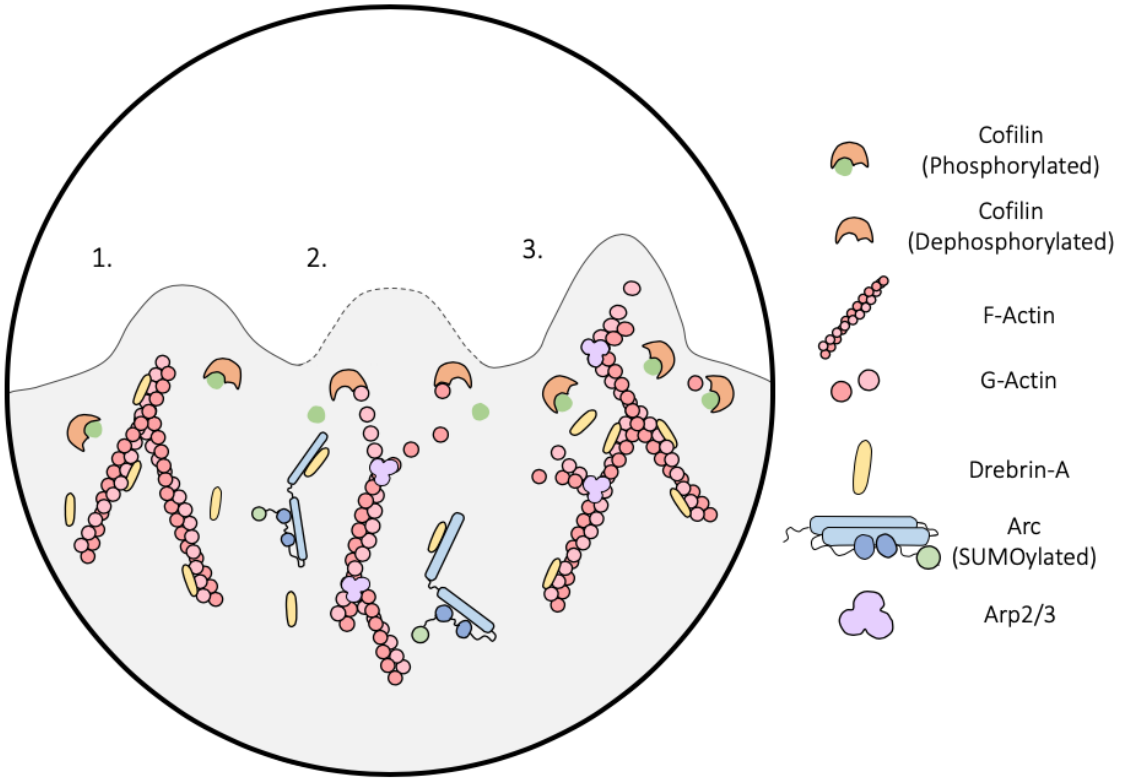


Figure 1-14 | Arc aids in growth of the cytoskeleton during plasticity events. 1) During basal stimulation, an equilibrium exists between F-actin growth and decay. Drebrin-A remains bound to– and stabilizes– f-actin, cofilin is phosphorylated and inactive. 2) Following stimulation, SUMOlyated Arc binds to, and releases, Drebrin-A from F-actin. This dissociation promotes dephosphorylation and activation of cofilin. Dephosphorylated cofilin is free to bind and destabilize F-actin. Severing and debranching of F-actin into monomeric G-actin promotes actin nucleation and elongation by increasing availability of filament ends, via Arp2/3. 3) Unbound from SUMOylated Arc, Drebrin-A returns to the spine, inhibits cofilin's actions and stabilizes the F-actin cytoskeleton.

Arc's ability to regulate and modify the actin cytoskeleton may in turn influence the formation and growth of spines. Increasing Arc expression in cultured hippocampal neurons has been shown to increase spine density by increasing the proportion of mature, thin and filopodia spines, decreasing the proportion of immature stubby spines (Peebles et al., 2010; Enriquez-Barreto et al., 2014). Conversely, Arc knockout mice show decreased spine density, further supporting Arc's role in activity regulated spine development (Huang et al., 2007; Bramham et al., 2008). Impairing Arc's ability to internalize GluA1-containing AMPAR, by overexpression of mutant Arc lacking the endophilin-3 binding domain (amino acids Δ91–100), failed to replicate Arc's increase in thin and filopodia spines. Therefore, this alteration in spine morphology may be driven, in part, by Arc's ability to simultaneously drive endocytosis of GluA1 AMPARs and regulate growth of the cytoskeleton (Peebles et al., 2010). Interestingly, previous work has shown that GFP-tagged *Arc* mRNA localizes stably and selectively to the base of stubby, thin, and mushroom spines by means of a mechanism which is not dependent on synaptic activity (Dynes and Steward, 2012). This may indicate that under conditions of basal activity docked *Arc* mRNA is selective for these spine types. Alternatively, synaptic activity has also been shown to simultaneously drive mRNA decay, eliminating *Arc* mRNA from inactive dendritic domains (Farris et al., 2014). This mechanism may allow or enhance the selective localization of *Arc* mRNA to active synapses, such that *Arc* mRNA may dock at the base of many spine types, but depending on the stimulation paradigm, mRNA is driven towards translation or degradation.

As well as localization within dendrites and regulation of the synaptic assembly, Arc has shown additional subcellular localization within the nucleus, concentrated in puncta associated with promolocytic leukaemia (PML) bodies, which are known to regulate gene transcription (Bernardi and Pandolfi, 2007). Here it has been proposed to regulate nuclear gene transcription (Eskiw and Bazett-Jones, 2002; Bloomer et al., 2008), involving the down regulation of GluA1 (G*ria1*) transcription (Korb et al., 2013). Thus, rapid upregulation of Arc may result in internalization of GluA1-containing AMPAR, in addition to reduced synthesis of GluA1 AMPAR subunits. Further, within the nucleus, Arc has been shown to interact with the histone-acetyltransferase, Tip60, a subunits of chromatin remodelling (Wee et al., 2014). This interaction has been shown to promote PML recruitment to enhance acetylation of histones. This finding indicates that Arc may also be involved in promoting learning induced gene-expression (Park et al., 2013). However, in response to acute cocaine administration, nuclear Arc expression appears to suppress chromatin remodelling and gene expression (Salery et al., 2017). These data may suggest that during periods of high activity, Arc acts primarily to reduce synaptic activity and gene expression, dampening down the network to maintain the expression of Hebbian plasticity.

Finally, while Arc has shown strong associations with AMPAR expression, and gene transcription, recent work has identified a novel role for Arc protein through shared properties with retroviral Gag proteins (Dodonova et al., 2019). Due to these similarities, Arc has been found to form virus-like capsids, capable of encapsulating and transporting functional RNA and protein between cells in endosomal-derived extracellular vesicles, in both cultured hippocampal rat neurons (Pastuzyn et al., 2018) and *Drosophila* neuromuscular junction (Ashley et al., 2018), therefore suggesting Arc can modulate synapse-, cell-, and network-wide plasticity.

1.6. Modulators of Plasticity

A final level in the dynamic control of plasticity includes molecules which act to modulate immediate synaptic transmission, or prime synapses for future strengthening or weakening. Much research has examined the mechanisms through which neurotrophins, hormones, and neuropeptides enhance the expression of LTP and LTD. In general, these modulators permit the synthesis, expression, or regulation of mediators and messengers of plasticity, but are not directly involved in the full expression of canonical LTP or LTD.

Here, I have defined neurotrophic molecules as modulators which share seemingly closely related mechanisms of action with a specific focus on the regulation of LTP and memory, and have been implicated in the pathophysiology of neurodegenerative disorders and neurological diseases. Based on previously established criteria (McAllister et al., 1999), Table 1-1 outlines a range of molecules with defined implications in plasticity. These criteria include:

1. Neurotrophic molecules and their cognate receptors must be expressed in the right places and at the right times for the form of synaptic plasticity being considered.

2. Expression and secretion must be activity dependent.

3. Must regulate aspects of neuronal function which change activity in neural circuits, including synaptic function, membrane excitability, and neuronal morphology and connectivity.

Table 1-1 | Identification of molecules involved in neuronal plasticity

Classification	*Abbreviation*	*Definition*	*Evidence for involvement in plasticity*
Neurotrophins	NGF	Nerve growth factor	(Sastry et al., 1988; Conner et al., 2009)
	BDNF	Brain-derived neurotrophic factor	(Cunha et al., 2010; Panja and Bramham, 2014; Solinas et al., 2019)
	NT-3	Neurotrophin 3	(Arvanov et al., 2000)
	Aβ	Amyloid-β	(Khan et al., 2010; Parihar and Brewer, 2010; Palmeri et al., 2017; Lazarevic et al., 2017 ; Gulisano et al., 2019)
	sAPPα	Secreted amyloid precursor protein α	(Clarris et al., 1994; Small et al., 1994;Ring et al., 2007; Young-Pearse et al., 2008; Taylor et al., 2008; Weyer et al., 2011; Hick et al., 2015; Klevanski et al., 2015; Richter et al., 2018; Mockett et al., 2019)
	sAPPβ	Secreted amyloid precursor protein β	(Furukawa et al., 1996b; Barger and Harmon, 1997; Chasseigneaux et al., 2011)
Neuropoietic factors	CNTF	Ciliary neurotrophic factor	(Stoop and Poo, 1996)
Hematopoietic factors	GCSF	Granulocyte colony-stimulating factor	(Diederich et al., 2009)
	IL-1, 2, 6, 11	Interleukins 1, 2, 6, 11	(Ross et al., 2003; Xiong et al., 2003; Chirila et al., 2014)
Growth factors	EFG	Epidermal growth factor	(Kopec et al., 2015),
	FGF	Fibroblast growth factors	(Wozniak et al., 2007)
	TNF	Tumour necrosis factors, α and β	(Stellwagen and Malenka, 2006; Liu et al., 2017)
Neuropeptides	ACTH	Adrenocorticotropic hormone	(Seidenbecher et al., 1993; Scantlebury et al., 2017)
	CGRP	Calcitonin gene-related peptide	(Wu et al., 2018a)
	CCK	Cholecystokinin	(Yasui and Kawasaki, 1995; Chen et al., 2019)
	CRF	Corticotropin-releasing factor	(Wang et al., 2000b; Blank et al., 2002)
	ENK	Enkephalin	(Bramham and Sarvey, 1996; Roberts et al., 1997)
	GAL	Galanin	(Sakurai et al., 1996; Badie-Mahdavi et al., 2005)

	NPY	Neuropeptide Y	(Whittaker et al., 1999; Sajdyk et al., 2008)
	NT	Neurotensin	(Amano et al., 2008)
	NRG1	Neuregulin-1	(Kwon et al., 2008; Jedlicka et al., 2015)
	SOM	Somatostatin	(Baratta et al., 2002; Chen et al., 2009)
	SP	Substance P	(Kato and Yoshimura, 1993; Dasgupta et al., 2017)
	VP	Vasopressin	(van den Hooff et al., 1989; Chafai et al., 2012)
	VIP	Vasoactive intestinal polypeptide	(Cunha-Reis et al., 2014)
Catecholamines, hormones	Ach	Acetylcholine	(Luo et al., 2008; Mitsushima et al., 2013; Takkala and Woodin, 2013)
	Epi	Epinephrine	(Kuba and Kumamoto, 1986; Kumamoto and Kuba, 1987)
	GH	Growth hormone	(Mahmoud and Grover, 2006; Molina et al., 2012)
	NE	Norepinephrine	(Izumi and Zorumski, 1999; Maity et al., 2015)
	DA	Dopamine	(Li et al., 2003; Swant and Wagner, 2006)
	5-HT	Serotonin	(Villani and Johnston, 1993; Hu et al., 2004; Mlinar et al., 2015)

1.6.1. *Brain-Derived Neurotrophic Factor*

One of the most acknowledged neuromodulators in the field of plasticity is the brain-derived neurotrophic factor (BDNF). BDNF belongs to a family of growth factors alongside nerve growth factor (NGF), neurotrophins-3, 4, 5 and 6 (NT-3-6). BDNF synthesis occurs through both activity-regulated and constitutive pathways. Interestingly, BDNF exists as one of the numerous mRNA transcripts present in the dendrites, and is capable of undergoing activity-dependent local translation (An et al., 2008). Similar to many neurotrophins, BDNF is initially synthesised as a precursor protein (preproBDNF) in the endoplasmic reticulum (Figure 1-15) Following cleavage, proBDNF is transported to the Golgi and sorted into either constitutive or regulated secretory vesicles (Greenberg et al., 2009). It is here that proBDNF can be converted into

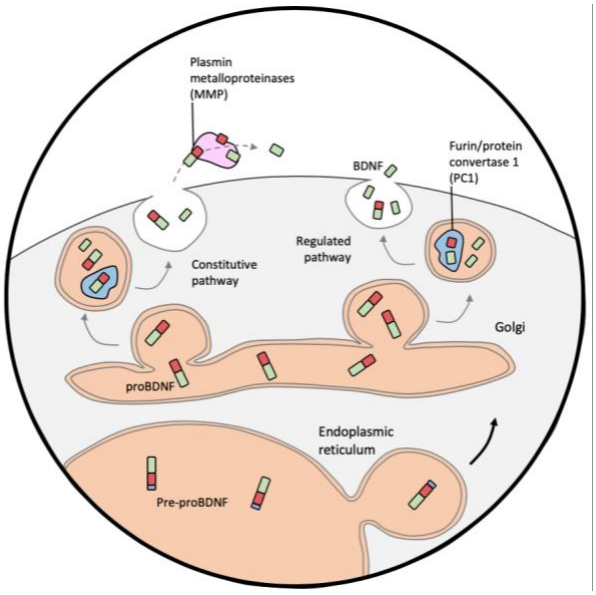


Figure 1-15 | Production and release of BDNF. Pre-proBDNF is synthesised and processed in the endoplasmic reticulum. ProBDNF is transported to the Golgi to be sorted. From here, ProBDNF is processed by furin or protein convertase 1 (PC1) into mature BDNF before release into the extracellular space or by plasmin metalloproteases (MMP) following release from the cell.

mature BDNF in the trans-Golgi network, by endoproteases such as furin, or in the immature secretory granules by proprotein convertases such as protein convertase 1 (PC1). Alternatively, following secretion proBDNF can be converted to mature BDNF by plasmin and matrix metalloproteinases present extracellularly.

BDNF acts through the tropomyosin receptor kinase B (TrkB), eliciting activation of downstream signalling cascades including Ras, MAPK, PKC, CaMKII, PI3K and PLC-γ (Sasi et al., 2017). Through these, BDNF mediates many neurotrophic processes, including neurogenesis (Lian et al., 2016; Liu and Nusslock, 2018), neuroprotection (Hofer and Barde, 1988), and cellular excitation and synaptic plasticity (Kang and Schuman, 1995; Kafitz et al., 1999). While proBDNF has been implicated in the facilitation of LTD (Woo et al., 2005), mature BDNF has been shown to enhance both E-LTP and L-LTP. Importantly, heterozygous and homozygous BDNF knockout mice show impaired maintenance of LTP (Korte et al., 1995), able to be rescued by virus-mediated perfusion of BDNF (Korte et al., 1996; Patterson et al., 1996). Interestingly, in conditions wherein the activity-dependent secretion of BDNF is inhibited, the maintenance, but not induction, of LTP is impaired (Sakata et al., 2013). While these past experiments have examined BDNF-mediated potentiation in response to tetanic stimulation, acute application of BDNF (25 minutes) to hippocampal slices has been shown to gradually enhance synaptic transmission in the dentate gyrus in the absence of tetanisation, or NMDAR activation (Ying et al., 2002). This enhancement of synaptic transmission was found to plateau within 3–4 hours post-BDNF, persisting without decrement for at least 15 hours. Further, this effect was found to be dependent on the early activity of MAPK, promoting CREB phosphorylation within 15 minutes and Arc transcription and translation within 3 hours (Messaoudi et al., 2002; Ying et al., 2002; Kuipers et al., 2016). Inhibition of BDNF by TrkB-targeted antibodies 45 minutes before the induction of LTP, or 10 minutes, 2- and 4-hours following the induction of LTP significantly attenuated the expression and maintenance of LTP *in vivo*. Application of TrkB-targeted antibodies 8- and 10-hours post-LTP induction did not significantly attenuate LTP persistence, indicating that BDNF may have an effective window in which it acts (Panja and Bramham, 2014).

In order to enhance the maintenance of LTP, it is thought that BDNF promotes the local synthesis of PRPs at the site of synaptic activity (Zhang and Poo, 2002; Leal et al., 2014). This mechanism has been described to mediate the synaptic tag and capture of PRPs during heterosynaptic plasticity. In conjunction with PKMζ, BDNF has been shown to enhance mGluR-primed LTP, converting a weak heterosynaptic LTP into a protein synthesis dependent, non-decaying LTP. Interestingly, although BDNF has been shown to coordinate a protein synthesis-dependent maintenance of LTP (Sajikumar and Korte, 2011), application of BDNF minutes after TBS stimulation has been shown to rescue an anisomycin-induced impairment in LTP maintenance (Pang et al., 2004).

This has been thought to occur through the protein synthesis-independent activation of PKMζ (Mei et al., 2011), suggesting PKMζ may act downstream of BDNF signalling.

Both pre- and postsynaptic mechanisms have been described in BDNF-mediated induction and maintenance of LTP. BDNF has been show to enhance presynaptic transmission (Gottschalk et al., 1998), likely by enhancing the synthesis of presynaptic vesicle proteins (Tartaglia et al., 2001), and the number of docked vesicles (Tyler and Pozzo-Miller, 2001). Postsynaptically, BDNF enhances the transcription, translation and trafficking of GluN1, GluN2A-, and GluN2B NMDAR (Caldeira et al., 2007), as well as the GluA1-containing CP-AMPAR (Smith et al., 2005; Li and Wolf, 2011; Fortin et al., 2012). The incorporation of CP-AMPAR has been shown to occur within 60 minutes, and was found to be dependent on mammalian target of rapamycin (mTOR), CaMKII, and protein synthesis (Caldeira et al., 2007).

1.6.2. *APP: A Multifaceted Control of Plasticity*

The APP protein is a lipid membrane bound protein 695-770 amino acids in length, belonging to the family of proteins including the amyloid precursor-like proteins (APLP1 and APLP2) in mammals and the amyloid precursor protein-like protein (APPL) in drosophila (Figure 1-16). APP exists in 8 isoforms, 3 of which are the most common: the APP695 is predominantly expressed in the CNS, while APP751 and APP770 forms, are more ubiquitously expressed (Bayer et al., 1999). Initially, full-length APP was understood to play a crucial role in regulating cell processes involved in metabolism and development, throughout the CNS, as well as kidney, heart, muscle, and lung tissue (Tanaka et al., 1989). APP knockdown mice show significant reductions in body weight, grip strength, locomotion and impaired synaptic transmission, as well as a susceptibility to epileptic seizures (Zheng et al., 1995). Further, in the CNS, APP is widely believed to be functionally involved in neuronal development, cell signalling, and homeostasis (Young-Pearse et al., 2007; O'Brien and Wong, 2011).

Within the CNS, APP is present in both excitatory and inhibitory neurons (Liao et al., 2016) at both pre- and postsynaptic terminals, as well as within glial cells including astrocytes and microglia (LeBlanc et al., 1996; Wang et al., 2009). Following synthesis and sorting in the ER and Golgi, APP is delivered to both axons (Koo et al., 1990) and dendrites (Das et al., 2013) by fast anterograde trafficking, mediated by kinesin-driven transport (Kamal et al., 2000). Following trafficking, both constitutive and activity-dependent processing permit cleavage of secreted metabolites (Figure 1-17). The processing of APP by α-, β-, and γ-secretases determines the outcome of the amyloidogenic versus the non-amyloidogenic pathway. Following trafficking from the trans-Golgi network, APP is transported to the cell surface. Here, APP may be proteolyzed directly by A disintegrin and metalloproteinase (ADAM10) α-secretase to generate the N-terminal secreted amyloid precursor

protein-α (sAPPα; Figure 1-16). Alternatively, a small population of cell surface APP may be processed by the β-site amyloid precursor protein-cleaving enzyme 1 (BACE1), releasing sAPPβ (Wang et al., 2018). Cleavage at the β-secretase site releases an APP fragment 16 amino acids shorter than sAPPα,

Figure 1-16 | Structure and domains of the Amyloid Precursor Protein. Schematic diagram depicts the APP protein and primary metabolites, as well as sites of α-(■), β-(■), γ-(■), ε-(■), and η (■)-secretase activity. Post-translational modifications include: phosphoserine (pS, ■), phosphothreonine (pT, ■), glycosylation (Gl, ■). Heparin-binding domain (HBD, ■), growth factor binding domain (GFBD, ■), Zn^{2+}- (■) and Cu^{2+}- (■) binding domains. E1 (■), ED (■), AD (■), E2 (■), juxtamembrane region (JMR, ■), transmembrane domain (■), APP intracellular domain (AICD; ■). Scale bar = amino acids. Structure based on (Nikolaienko et al., 2018).

and these 16 amino acids (CTα16) have been shown to mediate many of the differences between the two peptides (Richter et al., 2018; Morrissey et al., 2019b).

The dwell time of APP at the cell surface is short (Lorenzen et al., 2010). Unprocessed, full-length APP, or remaining C-terminal fragments following α-, and β-secretase activity (generating α and βCTFs, respectively), may be reinternalized in clathrin-coated pits into late endosomes or lysosomes containing both BACE1 and γ-secretase proteases. Here, unprocessed APP may undergo both β-secretase and γ-secretase processing to release sAPPβ, Aβ, and AICD, while α- and β-CTFs may also undergo further γ-secretase processing releasing p3, and APP intracellular domain (AICD), and Aβ, and AICD, respectively (O'Brien and Wong, 2011). Both p3 and AICD have been proposed to be involved in a range of intracellular signalling cascades, such as gene transcription, and neurite development, as well as caspase-mediated cell death signalling (Müller et al., 2008; Chow et al., 2010; Frykman et al., 2010; Zhou et al., 2012). This processing permits the production of sAPPα to preclude Aβ generation by cleavage and secretion at the plasma membranes (Nitsch et al., 1993; Lammich et al., 1999). Following generation of Aβ, Aβ fragments undergo lysosomal degradation or release into the extracellular space via vesicle recycling (Schroeder and Koo, 2005), or packaging into extracellular

vesicles (Sardar Sinha et al., 2018; Figure 1-17). Recently, alternative proteases have been identified which cleave APP to generate sAPPη (Willem et al., 2015) and CTF-ϵ (Eggert et al., 2004). Generation of the N-terminal product sAPPη by membrane-bound matrix metalloproteinases such as MT5-MMP (η-secretase) also releases CTF-η fragments Aηα and Aηβ, via α- and β-secretase, respectively. Conversely, the ϵ-secretase cleavage occurs in close proximity to γ-secretase and likely promotes release of the intracellular domain of APP following α, and β-secretase activity (De Strooper et al., 2010).

While processing of APP has been shown to occur constitutively, the release of both Aβ and sAPPα have also been linked directly to synaptic activity (Cirrito et al., 2003). Due to this, the naturally high metabolic activity of the hippocampus and closely associated cortices appear preferentially targeted for increased APP processing (Del Turco et al., 2016). During activity increased presynaptic vesicle fusion during neurotransmitter release, and clathrin-mediated endocytosis during vesicle recycling, promotes the delivery and internalization of APP, permitting Aβ generation and release. Alternatively, PKC-dependent activity has been shown to enhance cell surface expression of APP (Hung et al., 1993), promoting α-secretase activity, and inhibiting Aβ production (Skovronsky et al., 2000).

1.6.3. *Amyloid Beta*

Amyloid beta (Aβ) was first isolated as the principal component of amyloid deposits in the brain and cerebral vasculature of AD and Down's Syndrome (DS) patients (Masters et al., 1985). While Aβ has been shown to form peptides ranging from 39-43 residues in length, the primary forms are Aβ40 and Aβ42, produced by γ-secretase cleavage. It is through these two forms arise from poor specificity for the cleavage site (Li et al., 2000), while mutations in the γ-secretase complex protein PS1 (*PSEN1*) drive γ-secretase towards Aβ42 production (Wolfe, 2007). The presence of the longer C-terminal tail of Aβ42 is thought to promote aggregation into higher order molecules, and the formation of extracellular Aβ plaques (Jarrett et al., 1993). Due to this, a consistent feature of AD pathology is an increased ratio of Aβ42/40, suggesting a shift in balance of these products is a critical step in AD pathogenesis

Aβ has been widely understood as a key regulator of the neurodegeneration observed in AD. The presence of extracellular Aβ-containing plaques and intracellular hyperphosphorylated Tau protein and accumulation of neurofibrillary tangles (Spillantini et al., 1990). These dystrophies are thought to occur downstream of aberrant Aβ signalling, promoted by sporadic or genetic factors contributing to the pathogenesis of AD (Ricciarelli and Fedele, 2017). These changes, mediated in

large by Aβ, have been linked to the loss of excitatory synapses (Lue et al., 1999), impairments in memory and LTP (Shankar et al., 2008; Faucher et al., 2016), and neuritic dystrophy (Pike et al., 1992), ultimately leading to cell death (Bancher et al., 1997) and atrophy of the CNS (Whitwell, 2010). Notably, in animal models expressing mutated forms of APP or PS1, deficits in synaptic composition and transmission are present before the emergence of plaques by several months (Hsia et al., 1999). At the synapse, oligomeric Aβ has been shown to bind in close proximity to synapses (Lacor et al., 2004; Koffie et al., 2009), and impair synaptic density in a radial manner from Aβ plaques (Hsieh et al., 2006; Spires-Jones et al., 2007; Birnbaum et al., 2015). These oligomers have been further shown to impair the induction and maintenance of LTP (Freir et al., 2001; Shankar et al., 2008), enhance LTD (Li et al., 2009), and reduce cell surface levels of GluN1 and GluN2B NMDAR subunits (Snyder et al., 2005; Kurup et al., 2010), as well as synaptic GluA1, (Almeida et al., 2005), and GluA2 (Liu et al., 2010) AMPAR subunits. From this, it is posited that small Aβ oligomers provide a majority of the neurotoxicity evident in AD, with Aβ plaques providing diffuse stores of these peptides.

This research provides strong evidence that Aβ acts to promote pathological dysregulation of synapses, however under conditions in which high concentrations of Aβ are present. Converse to this, a non-pathological role of Aβ has also been identified at lower, physiological levels. At concentrations reflective of those present in the healthy brain (Rozmahel et al., 2002; Phinney et al., 2003; Pawlik et al., 2004), mounting evidence suggest that Aβ shows characteristics of a canonical neurotrophin. Such that APP, and thus Aβ, as well as β- and γ-secretases are expressed throughout the hippocampus (Hébert et al., 2004; Del Turco et al., 2016; Meakin et al., 2018) and cortex (Satoh et al., 2012; Bergström et al., 2016), from early in development throughout adulthood (Yusof et al., 2019; Bergström et al., 2016). Further, as mentioned above, the generation of Aβ is activity dependent, with extracellular release tightly linked to vesicle fusion and recycling (Cirrito et al., 2003), and Aβ has shown promise in positively modulating neuronal function, such as enhancing synaptic transmission, cell excitability, and promoting plasticity.

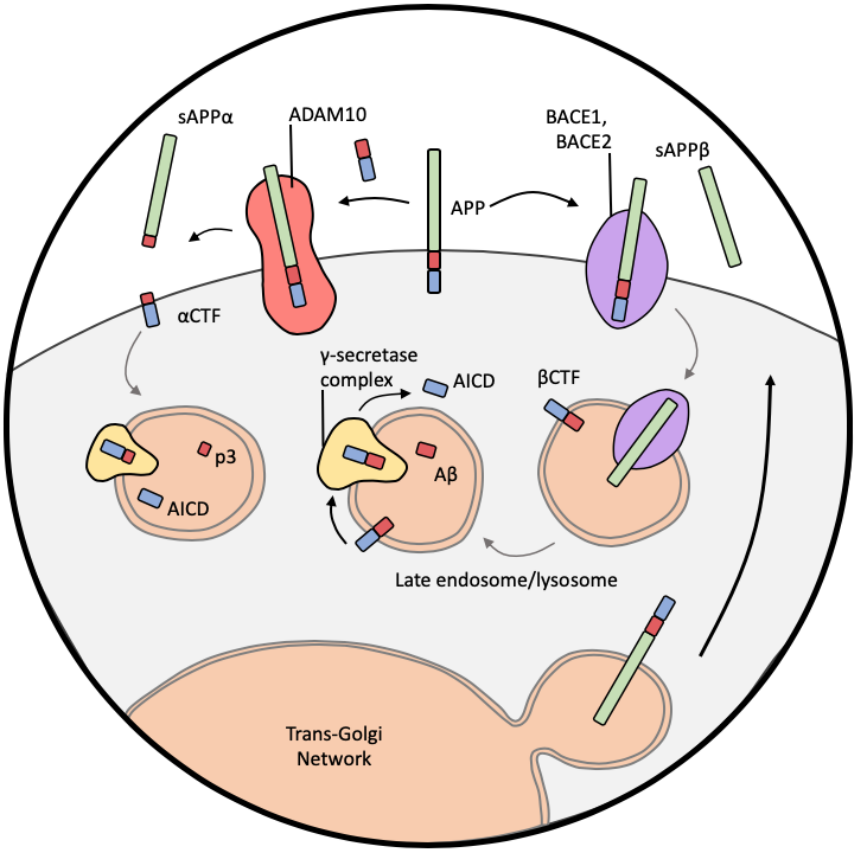


Figure 1-17 | Stylized diagram of APP processing. In the non-amyloidogenic pathway, APP is trafficked towards the plasma membrane where it is cleaved by α-secretase, generating soluble APP alpha (sAPPα) and α-secretase C-terminal fragment (α-CTF). Full length APP is internalised by clathrin-dependent endocytosis. APP, BACE1 and BACE2 converge in early endosomes. The amyloidogenic pathway then results in APP cleavage into soluble APPβ (sAPPβ) and β-secretase C-terminal fragment (β-CTF; also known as C99). Both non-processed, full-length APP and sAPPβ can be reinserted into the plasma membrane through recycling endosomes. Final γ-cleavage of APP generates the APP intracellular domain (AICD) and amyloid β (Aβ) peptides.

It has been previously shown that inhibition of Aβ degradation enhances mEPSC responses through an enhancement in presynaptic vesicle release (Abramov et al., 2009; Lazarevic et al., 2017). Likewise, picomolar concentrations of Aβ enhances neuronal neurotransmission (Puzzo et al., 2008), and spontaneous astrocytic calcium transients (Lee et al., 2014), both in a manner dependent on the activation of α7nAch receptors. Further, cGMP-induced LTP has been found to be dependent on Aβ through a mechanism which increases APP/BACE-1 convergence in lysosomes (Palmeri et al., 2017). In line with this, recent work has highlighted a role of Aβ in the conversion of E-LTP to a protein synthesis-dependent L-LTP, as well as increasing phosphorylation of CREB and CaMKII and increasing BDNF levels, in a manner dependent on the NO/cGMP/PKG cascade and α7nAchR activation (Gulisano et al., 2019). Interestingly, Aβ may act in a concentration- and aggregation-dependent manner to gate α7nAchR activity. Such that, at picomolar concentrations, monomeric Aβ promotes α7nAchR

activation (Dineley et al., 2002; Dougherty et al., 2003; Wu et al., 2007; Puzzo et al., 2008), while nanomolar concentrations (Liu et al., 2001; Pettit et al., 2001; Grassi et al., 2003) and oligomeric Aβ (Kroker et al., 2013) inhibit the activation of α7nAchRs (Wang et al., 2000a; Lasala et al., 2019). Lastly, depletion of Aβ *in vivo* has been shown to result in impaired performance on both reference and fear conditioning-based tasks (Puzzo et al., 2008; Garcia-Osta and Alberini, 2009). Taken together, these data provide evidence for a role of Aβ in regulating memory formation at physiological concentrations, while significantly impairing synaptic plasticity and neuronal health at greater concentrations (Figure 1-18). These dual mechanisms displayed by Aβ may provide insight into why many of the drug treatments aiming to reduce or eliminate Aβ in AD fail (Lista et al., 2015; Morley and Farr, 2016).

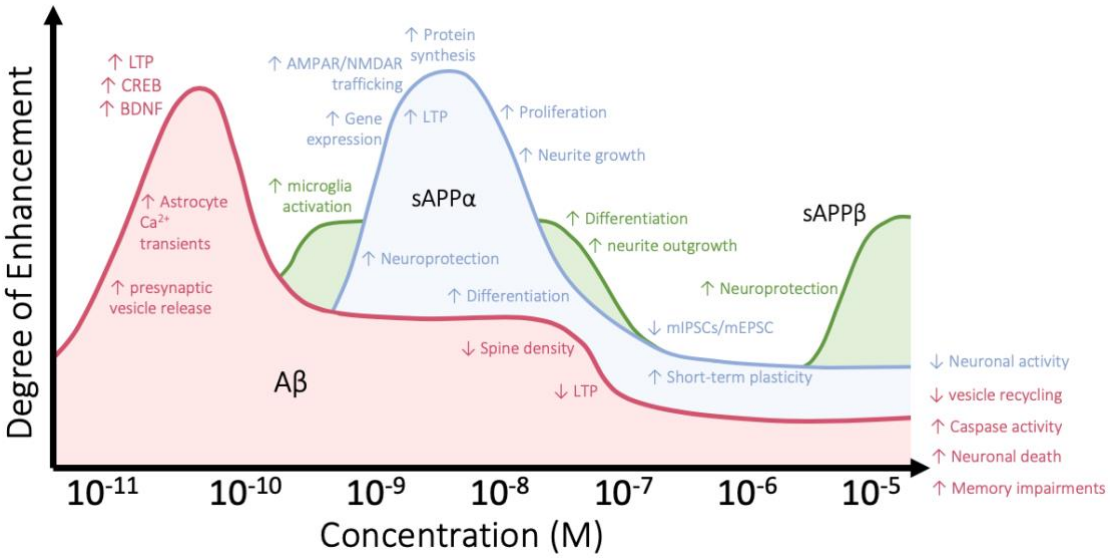


Figure 1-18 | Hormetic relationship between APP metabolites and the expression of key neurotrophic mechanisms. Aβ (■), sAPPβ (■), and sAPPα (■) show distinct concentration-dependent regulation of cellular processes, with minimal overlap. Many of the positive effects of Aβ- and sAPPα are present at low picomolar and nanomolar concentrations, respectively. sAPPα begins to inhibit neuronal active at high nanomolar concentrations, while Aβ becomes detrimental to plasticity and cell health at high nanomolar and micromolar concentrations. Effective sAPPβ concentrations appear at low nano- and micromolar concentrations. Concentrations are presented as a log of molar (M) concentration.

1.6.4. *sAPPα*

A facet of the APP protein which has arisen throughout the course of its research is its role in promoting neurotrophic support during development, as well as contributing to the regulation of plasticity. Research examining acute and chronic application or knockout of APP have provided

significant evidence that APP mediates many aspects of central and peripheral nervous system function. Initial observations found that knockout of APP resulted in reduced peripheral motor function (Zheng et al., 1995; Zheng et al., 1996; von Koch et al., 1997), defective CNS neuronal migration (Herms et al., 2004), reduced neuronal morphology and synaptic transmission (Zheng et al., 1995; Zheng et al., 1996; Dawson et al., 1999), and impaired spatial memory performance (Müller et al., 1994). Around this time the N-terminus of APP was identified to contain growth factor-like structure (Rossjohn et al., 1999), containing heparin-binding, and cysteine-rich domains. Animal models lacking the expression of N-terminal APP, but retaining the amyloidogenic C-terminal domain, show extensive gliosis, impaired spatial memory performance, and reduced LTP magnitude (Nalbantoglu et al., 1997). Importantly, many of the deficits displayed by knockout or mutation of the endogenous APP gene, could be rescued with acute application of sAPPα, restoring brain and body weight, as well as motor deficits and synaptic transmission and spatial memory (Ring et al., 2007). Importantly, application of sAPPβ was insufficient to rescue the increased lethality of APP-KO mice, nor the associated motor deficits (Li et al., 2010). While sAPPβ has been shown to regulate aspects of neurite outgrowth (Chasseigneaux et al., 2011), neural differentiation (Freude et al., 2011), and neuroprotection (Furukawa et al., 1996b; Barger and Harmon, 1997), mounting evidence suggests sAPPα mediates many of the neurotrophic and plasticity-enhancing effects present in APP, at lower effective concentrations (see Figure 1-18). Crucially, in many neurological and neurodegenerative disorders, the expression and regulation of sAPPα appears altered and may contribute significantly to the pathology of the disease.

1.6.4.1. *sAPPα as a Biomarker*

As part of the pathology which arises in AD and associated pathologies, the amyloid cascade hypothesis further posits that there is a clear shift in the processing of APP towards the amyloidogenic pathway, and away from the non-amyloidogenic pathway (Selkoe and Hardy, 2016). AD is a multifactorial disease, consisting of both familial (fAD) and sporadic (sAD) forms (Barykin et al., 2017). A minority of AD cases present with fAD, with a predominantly early-onset, driven primarily by the inheritance of mutations in *APP*, *ADAM10*, *PSEN1*, and *PSEN* (Bertram et al., 2010). Most cases (90%) present as the more complex sAD, arising from a combination of environment and lifestyle factors (Piaceri et al., 2013), as well as genetic predisposition associated with the ε4 allele of the Apolipoprotein E gene (*APOE*), in approximately 20% of sAD cases (Ertekin-Taner, 2010). *APOE* has been described to regulate the metabolism and clearance of Aβ from the CNS (Verghese et al., 2013), and therefore has been implicated in contributing to Aβ-mediated pathology. Importantly, both fAD

and sAD have been hypothesised to arise, in part, as a result of the amyloid cascade hypothesis. Such that mutations which directly influence APP processing in fAD or Aβ clearance in sAD alter Aβ expression in the CNS. Logically, if a therapeutic is administered before the deposition and accumulation of Aβ, which opposes the further deposition of Aβ or counteracts early dysfunction, a therapeutic such as this would be expected to delay onset or severity of the disease. However, a caveat of this arises such that the period before detrimental cognitive decline, but possible to be detected physiologically, must be defined.

Due to this, many studies have attempted to measure the metabolites of APP processing, including sAPPα, sAPPβ, total sAPP, and Aβ40/42, from blood, plasma, and cerebral spinal fluid (CSF) from human patients, aiming to detect onset or manifestation of neurodegenerative diseases, including AD. Much of the research examining the expression of APP metabolites in AD has focused on Aβ, finding strong correlations between levels of Aβ40/42 with Mini-Mental State Examination (MMSE) scores (Struyfs et al., 2015) and disease progression (Bibl et al., 2012; Janelidze et al., 2016), in both sAD (Portelius et al., 2010) and fAD (Portelius et al., 2012). Studies examining the concentration of sAPPα in AD patients have however been somewhat inconsistent. This may have arisen partially through discrepancies in the methodology, including MMSE scores, heterogeneity of the disease, comorbid conditions, specificity and sensitivity of the assays and cross-reactivity of antibodies, and differences in sampling, processing, and storage of samples. One study in particular was conducted on patients scoring greater than 20 on the MMSE. Of those patients with CSF characteristics of AD, many demonstrated high CSF levels of sAPPα and sAPPβ (Lewczuk et al., 2010). However, comparing this to studies which have found a significant decrease in sAPPα while using less strict exclusion criteria, the increased levels of APP metabolites may be indicative of patients with typically higher cognitive ability (scoring > 20) despite presence of pathological AD characteristics, or those in an early stage of the disease progress (Prior et al., 1991; Van Nostrand et al., 1992; Sennvik et al., 2000; Colciaghi et al., 2002; Rosen et al., 2012). Notably, in studies which concluded little to no change in AD patients CSF levels of sAPPα and sAPPβ, the antibodies used failed to distinguish between sAPPα and sAPPβ and thus measured total sAPP (Palmert et al., 1990; Hock et al., 1998). This limits the conclusions drawn as many recent studies suggest unchanged sAPPβ with decreased sAPPα (Prior et al., 1991; Van Nostrand et al., 1992; Sennvik et al., 2000).

Furthermore, differences may arise in the aetiology of the disease. In patients with fAD mutations such as those affecting APP (Swedish APP KM670/671NL) and ADAM10 (Q170H and R181G), there are significant decreases in CSF levels of sAPPα (Lannfelt et al., 1995; Kim et al., 2009), which is further correlated with poor performance on neuropsychological tests that assess intelligence, verbal and visuospatial functions, memory, and attention (Almkvist et al., 1997). A similar

trend is seen in patients possessing one or two copies of the ε4 allele of APOE, suggesting risk factors for sAD may also affect the processing and expression of sAPPα (Olsson et al., 2003; Thordardottir et al., 2017). Importantly, while the levels of sAPPα significantly decrease in fAD, and in moderate-to-severe AD (Lannfelt et al., 1995; Almkvist et al., 1997), it appears many studies have not found significant changes in the early stages of sAD and mild cognitive impairment (MCI; Perneczky et al., 2011; Rosen et al., 2012; Brinkmalm et al., 2013; Perneczky et al., 2013; Araki et al., 2017), compared to sAD patients in advanced stages of the disease (Rosén et al., 2012). This indicates that direct genetic alterations in APP processing may influence biofluid levels of sAPPα to a greater, more detectable level than in sAD. Interestingly, reduced CSF concentrations of sAPPα are also evident in other conditions, including cerebrovascular and neurodegenerative diseases such as stroke (Selnes et al., 2010), amyotrophic lateral sclerosis (ALS; Selnes et al., 2010; Steinacker et al., 2011), bipolar disorder (Jakobsson et al., 2013; Rolstad et al., 2015), and idiopathic normal pressure hydrocephalus (Miyajima et al., 2013), therefore changes in the levels of sAPPα in these conditions may indicate a critical clinical evaluation is necessary to rule out comorbid diseases which may interfere with the specificity of an AD diagnosis.

1.6.4.2. *sAPPα as a Neuroprotective Molecule*

Following the observations that APP can regulate copper- (White et al., 1999), iron- (Maynard et al., 2002; Duce et al., 2010), zinc-, and manganese- (Needham et al., 2014) homeostasis, levels of glucose and insulin (Needham et al., 2008), as well as provide anticoagulant functions with regards to thrombosis (Henry et al., 1998; Xu et al., 2009), it was becoming clear that sAPPα may also demonstrate neuroprotective and neurotrophic functions. sAPPα has since been shown to protect against a battery of insults including traumatic brain injury (TBI; Pierce et al., 1996; Chen et al., 2004; Thornton et al., 2006; Loane et al., 2009; Corrigan et al., 2012; Siopi et al., 2013; Plummer et al., 2016), hypoglycemia and glutamate toxicity (Mattson et al., 1993; Furukawa et al., 1996b; Ryan et al., 2013), and proteasomal deficiency-induced cell stress and apoptosis (Copanaki et al., 2010; Kundu et al., 2016). While many of these assays examine sAPPα's ability to counteract apoptosis or apoptosis-related pathways, a 2016 study examined the effects of transient hypoxia on electrical activity at the network and cellular level, in APP-KO mouse tissue. Relative to wild-type (WT) mice, in APP-KO mice hypoxia-induced impairments were exacerbated, including increased intracellular Ca^{2+}, faster loss of function, higher incidence of spreading depression, and much slower recovery upon reoxygenation (Hefter et al., 2016). Importantly, these deficits could be abrogated following selective expression of sAPPα or blockade of L-type calcium channels. These results indicate that sAPPα contributes much of

its neuroprotective functions to calcium homeostasis. Of interest, many of the neuroprotective effects including activation of nuclear factor-kappa B (NFκB) DNA-binding activity, calcium homeostasis, and K^+-channel activation, occur as part of the cGMP pathway (Furukawa et al., 1996a; Furukawa and Mattson, 1998). Further, *in vivo* studies have corroborated this research, finding that during transient hippocampal ischemia, administration of sAPPα significantly ameliorated neuronal loss (Smith-Swintosky et al., 1994), consistent with findings that APP-KO and BACE-KO mice show increased mortality as a result of cerebral ischemia (Koike et al., 2012). Similarly, APP-KO mice appear more vulnerable to TBI, but show reduced cell death, and axonal deficits, and improved motor outcomes following intracerebroventricular administration of sAPPα or peptides derived from it (Corrigan et al., 2014), indicating an endogenous role of sAPPα during injury. Importantly, sAPPα has also been shown to provide neuroprotection against Aβ-induced toxicity (Goodman and Mattson, 1994; Barger and Mattson, 1996b; Gralle et al., 2009; Milosch et al., 2014), by promoting an upregulation in neuroprotective genes such as transthyretin and insulin-like growth factor-2 (Stein et al., 2004). sAPPα may also directly regulate the expression of Aβ, by binding to and inhibiting of BACE1 directly, reducing the production of Aβ (Obregon et al., 2012; Peters-Libeu et al., 2015). These observations further support the hypothesis that dysregulation of APP processing exacerbates Aβ-mediated pathology through an impairment in sAPPα-mediated neuroprotection.

1.6.4.3. *sAPPα as a Putative Treatment*

The rise of the amyloid cascade hypothesis has largely driven by efforts to minimize levels of Aβ in the brain. This has led to clinical trials aimed at reducing Aβ action directly or inhibiting its production. Among the anti-Aβ therapeutic approaches, the most extensively developed is that of immunotherapy– direct targeting of endogenous Aβ protein through administration of exogenous monoclonal antibodies (van Dyck, 2018). While many attempts have reached phase 1 (Ostrowitzki et al., 2012; Landen et al., 2013; Sevigny et al., 2016) or 2 (Salloway et al., 2009; Rinne et al., 2010; Farlow et al., 2012) of clinical trials, many have also made it through to phase 3 (Doody et al., 2014; Salloway et al., 2014). However, all cases have failed to meet their primary end points, with no significant changes to disease progression or cognition in the face of major problems with safety. Because of this, indirect targets of Aβ generation have been sought. The β-secretase enzyme BACE1 has been shown to be significantly increased in AD patients (Fukumoto et al., 2002; Fukumoto et al., 2004), and significantly correlated with Aβ load (Li et al., 2004), and has therefore been targeted as a target of potential therapeutics. Early investigations found BACE1 inhibitors to provide significant ameliorations to brain Aβ levels (Lai et al., 2012; Stamford et al., 2012; Bernier et al., 2013; Forman et

al., 2013; Eketjäll et al., 2016) while increasing sAPPα levels (Martenyi et al., 2012; Willis et al., 2012). However, many of these trials failed to provide significant ameliorations in cognitive or functional decline (Hawkes, 2017; Egan et al., 2018), likely due to little benefit in reducing Aβ late in the disease progression, and off-target effects (Lahiri et al., 2014).

Due to sAPPα's innate role in combating neurodegeneration, whilst providing trophic support, it is only logical to approach treatment against neurodegeneration from the standpoint of enhancing either direct CNS concentrations of sAPPα, or upregulating endogenous mechanisms which do so. Perhaps surprisingly, recent investigations into FDA-approved drugs marketed towards improving symptoms present during mild-moderate AD, has shown a unique link to APP physiology. Cholinesterase inhibitors Rivastigmine, Phenserine, and Donepezil have been show to significantly decrease Aβ *in vitro* (Lahiri et al., 2007; Takada-Takatori et al., 2019), *in vivo* (Ray et al., 2020), and in CSF of AD patients (Maccecchini et al., 2012). Additionally, Rivastigmine has been found to inhibit Aβ generation through a mechanism which enhances ADAM-9, -10, and -17 α-secretase activity, and thus simultaneously enhance sAPPα production. This effect was found to be robust when assayed in rat neuronal PC12 cells, primary human brain cultures, 3× transgenic (APPKM670/671NL, PS1M146V, MAPTP301L) mice, as well as post-mortem human brain samples from those with AD and treated with Rivastigmine. The action of these drugs is spurred by the cholinergic hypothesis of AD, which posits that cholinergic hypofunction mediates the cognitive decline present in AD, and augmentation of this may be beneficial, by either enhancing activity of the endogenous acetylcholine (ACh) or by exogenous agonists (Fisher, 2012). Results from animal research provides support for this as high concentrations of Aβ impair α7nAchR activation (Liu et al., 2001; Pettit et al., 2001; Grassi et al., 2003), which negatively affect the induction of LTP (Kroker et al., 2013), but can be rescued by co-application of competitive agonists of α3β4 (Nery et al., 2013) and α4β2- and α7nAchRs (Kroker et al., 2013). Importantly, activation of α4β2-, and α3- and α7-containing nAchR (Nitsch et al., 1992; Mousavi and Hellstrom-Lindahl, 2009), as well as m1- and m3-containing muscarinic AChRs (mAchRs; Nitsch et al., 1996), enhances the release of sAPPα while simultaneously attenuating Aβ production, indicating that Aβ itself may drive positive feedback regulation at physiological levels and may be an avenue worth exploring therapeutically.

1.6.4.4. *sAPPα as a Promoter of Plasticity*

In addition to promoting neuroprotection, APP has been shown to contribute to the growth of neurons, promoting the formation and strengthening of synapses and enhancing plasticity. APP-null and APP-silenced mice show significant reductions in the proportion of synaptophysin-positive

presynaptic terminals, impaired induction and maintenance of hippocampal LTP (Seabrook et al., 1999; Taylor et al., 2008), in addition to prominent reductions in total neurite length, dendritic branching, reduced spine density and reduced spine head volume (Ring et al., 2007; Hick et al., 2015). As research has continued, sAPPα has begun to prove itself as not only a key facilitator of neuroprotection but as a neurotrophic and plasticity-enhancing protein, also. Early observations found that APP-null and APP-silenced (through antibody targeting) mice showed prominent reductions in the proportion of synapses (Seabrook et al., 1999), reductions in total neurite length, dendritic branching, reduced spine density and reduced spine head volume (Lee et al., 2010b; Tyan et al., 2012), as well as significant reductions in the induction and persistence of hippocampal LTP (Ring et al., 2007; Weyer et al., 2011; Hick et al., 2015), and impaired performance on hippocampal-dependent memory tasks (Klevanski et al., 2015). Importantly, many of these impairments are able to be rescued by acute application or viral knock-in of sAPPα or the C-terminal 16 amino acid APP672–688 peptide (CTα16), but not the sAPPβ fragment (Ring et al., 2007; Weyer et al., 2011; Hick et al., 2015; Klevanski et al., 2015), indicating that many of the synaptic functions provided by APP may arise by sAPPα-driven mechanisms. In fact, sole application of sAPPα to hippocampal tissue has been shown to promote neurite (Clarris et al., 1994; Small et al., 1994), and axonal outgrowth (Young-Pearse et al., 2008), primarily produced through the presence of heparin-binding site at residues APP96-110 (Small et al., 1994), APP319–335 containing the amino sequence RERMS (Ninomiya et al., 1994), or the APP328-332 motif alone (Jin et al., 1994). In line with this, overexpression of human APP (Mucke et al., 1996; Lee et al., 2010b), or the α-secretase ADAM10 (Bell et al., 2008) has been shown to increase the proportion of cholinergic, glutamatergic and GABAergic synapses, accompanied by increases in surface and total GluA2, but not GluA1-containing AMPAR (Lee et al., 2010b). Further, using time-lapse two-photon in vivo microscopy, spine density was found to remain unaltered in APP-KO mice, however these mice showed significant deficits in spine turnover, affecting spine formation and elimination, as well as environmental enrichment-dependent increase in spine density. Interestingly, the latter was found to be rescued by application of exogenous D-serine (Zou et al., 2016), indicating that APP, or sAPPα may mediate astrocyte and D-serine homeostasis to regulate these changes. Together, these results indicate a crucial role of autocrine or paracrine signalling of sAPPα in the formation and maintenance of spines, necessary for learning-induced plasticity.

In addition to altering morphological plasticity, sAPPα has been shown to also facilitate functional physiological outcomes. Early experiments showed that application of sAPPα to acute hippocampal slices shifts the frequency of the LTD induction, such that low frequency, LTD-inducing stimulation (LFS, 1Hz) was insufficient to induce LTD following pre-treatment with sAPPα. Alternatively, sAPPα was also shown to enhance HFS-induced (100Hz) LTP by 50% (Ishida et al., 1997),

in a manner dependent on cGMP, and likely PKG. Similarly, inhibition of endogenous sAPPα by antibody targeting, or inhibition of α-secretase by TAPI-1, significantly reduces LTP in the dentate gyrus of adult rats *in vivo* (Taylor et al., 2008), as well as impairing spatial memory. These results have been so-far linked to an increase in NMDAR currents (Moreno et al., 2015), however this is contested (Furukawa and Mattson, 1998), while more recent work has linked the activation of α7nAChRs in sAPPα- and CTα16-mediated enhancements of LTP (Richter et al., 2018; Morrissey et al., 2019a).

sAPPα's ability to enhance synaptic potentiation, in both *in-* and *ex-vivo* preparations, is further evident by sAPPα's effect on behaviour. Intracerebral administration of antibodies targeted towards residues 672–688 of APP has been shown to impair performance on inhibitory avoidance in rats (Doyle et al., 1990), and passive avoidance in chicks (Mileusnic et al., 2000). Similarly, inhibition of α-secretase impairs spatial water-maze memory in rats (Taylor et al., 2008), similar to complete knockout of APP (Ring et al., 2007). Importantly, these deficits could be restored by either acute administration (Taylor et al., 2008) or genetic overexpression (Ring et al., 2007) of sAPPα. Further, these memory-enhancing effects have been found to be applicable in rescuing amnesia- (Meziane et al., 1998), Aβ- (Mileusnic et al., 2004), AD- (Fol et al., 2016), TBI- (Corrigan et al., 2012), and aging-induced memory impairments (Xiong et al., 2017). Further, acute treatment of sAPPα, but not sAPPβ, is capable of protecting against oligomeric Aβ-induced spine loss (Tackenberg and Nitsch, 2019), while both sAPPα and CTα16 have been shown to rescue impairments in LTP in an AD mouse model (Fol et al., 2016; Morrissey et al., 2019a). Interestingly, evidence from human studies, show in both AD and Bipolar Disorder (BD), cerebrospinal fluid concentrations of sAPPα have been found to be significantly lower than healthy controls (Zhang et al., 2011; Jakobsson et al., 2013; Rolstad et al., 2015). In addition, decreased sAPPα is significantly correlated with decreased cognitive performance on attention and speed in those with BD.

While many strides have been made in understanding how sAPPα promotes LTP and concomitant modulations to memory and behaviour, many of the underlying mechanisms remain unsubstantiated. sAPPα has been previously shown to enhance both *de novo* transcription and translation (Ryan et al., 2013; Mockett et al., 2019), with transcription thought to be mediated through NFκB (Barger and Mattson, 1996a). The outcomes of this appear to mediate extensive gene expression, much of which regulates neuroprotection through cell survival, inflammatory, anti-apoptotic, and neurogenic responses (Stein et al., 2004; Aydin et al., 2011; Demars et al., 2011).

sAPPα has been further shown to regulate translational processes by directly regulating protein synthesis. Application of sAPPα (1, 10 nM; 25 min) but not sAPPβ has been shown to enhance

synaptodendritic protein synthesis in rat hippocampal synaptoneurosomes (Claasen et al., 2009), independent of transcription. This effect was found to be dose-dependent and dependent on the activity of CaMKII, MAPK, and PKG. Interestingly, the degree of protein synthesis enhancement was significantly affected by age, such that old (22–23-month-old) rats increased protein synthesis approximately 15% less than young (8-12 weeks) counterparts. More recently, sAPPα has been shown to enhance LTP in a concentration- and protein synthesis-dependent manner (Mockett et al., 2019) as well as requiring trafficking of proteins from the Golgi to the cell surface. Importantly, this enhancement of LTP was found to be specific to sAPPα, as LTP remined unaffected by sAPPβ. Further, sAPPα was found to concomitantly increase cell surface levels of GluA1 but not GluA2 AMPAR subunits, in a concentration (0.3, 1 nM)-dependent manner and dependent on CaMKII, PKG and activation of NMDAR. Similarly, sAPPα also increased cell surface levels of GluN1, in a concentration (1 nM)- and CaMKII-dependent manner. Further, in primary hippocampal cell culture, sAPPα (1 nM, 2 hr) was found to enhance *de novo* synthesis of GluA1, but not GluA2 AMPAR subunits (Mockett et al., 2019). Interestingly, the enhancement of LTP is able to be recapitulated by application of as little as three amino acids found within the E2 APP328-330 domain of sAPPα (Morrissey et al., 2019a), referred to as the RER peptide, as well as CTα16 (Morrissey et al., 2019b), in a concentration- and protein synthesis-dependent manner.

Together, these results define many likely mechanisms through which sAPPα, and associated peptides, may promote the expression of synaptic plasticity and memory. This work has established gene expression, protein synthesis, and glutamate receptor trafficking as key processes mediating these changes, yet the underlying details remain largely unknown. To date, no study has examined the effect of sAPPα on the expression of IEGs such as Arc and the relationship to cell surface expression of AMPAR. The regulation of these processes is critical to the expression of LTP, and therefore would provide important insights into the regulation of memory in both health and disease. This thesis aims to extend the current understanding of the mechanisms underlying sAPPα-mediated plasticity by investigating AMPAR trafficking and Arc protein in hippocampal neurons. We hypothesise that sAPPα regulates the enhancement of LTP, in part through the insertion of newly synthesised GluA1-containing CP-AMPAR at the cell surface. Moreover, we hypothesise that the expression of these AMPAR would be regulated by the expression and function of the IEG Arc.

Chapter 2: Materials and Methods

2.1. Primary Neuronal Culture Preparation

All experimental protocols conducted in New Zealand were approved by the University of Otago Animals Ethics Committee and conducted in accordance with New Zealand Animal Welfare Legislation under the ethics approval ET18/15.

The preparation of primary hippocampal cultures followed a modified protocol based on Banker and Goslin, (1998), Banker and Cowan, (1977) and Kaech and Banker, (2006), and refined by Dr. Tet-Woo Lee and Dr. Megan Elder. Antibiotics were not used for experiments examining the expression of glutamate receptors but were present for the examination of Arc protein. All instruments were autoclaved and all liquids were filter sterilized prior to use. Aseptic techniques were employed at all times. Glass bottom culture dishes (50 mm, 14 mm glass, MatTek Corporation, #P35G-1.5-14-C), 6 well plastic dishes (Corning, #3516), 96-well assay plates (Corning, #3603), or 175 cm^2 flasks (Sigma, #CL55431306) were coated overnight with Poly-D-Lysine (PDL; 100-200 µg/mL, Sigma), rinsed with autoclaved sterilised Milli-Q ultrapure water (MQ), and left to dry in a Herasafe KS Class II Biological Safety Cabinet (Thermofisher).

2.1.1. *Dissection*

Two Sprague-Dawley rat pups (male or female, postnatal day (PD) 0-1) were sourced from a breeding colony maintained at the Hercus Taieri Resource Unit by the University of Otago. Pups were decapitated with a sharp pair of scissors (Fine Science Tools, #14060-09), and heads were submerged in a 100 x 20 mm petri dish (Corning, #430591) containing ice-cold dissection media (DM; in mM: 82 Na_2SO_4, 30 K_2SO_4, 5.8 $MgCl_2$, 0.252 $CaCl_2$, 1 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 20 glucose, 0.001% Phenolred in MQ, adjusted to pH 7.4) and moved to a sterile laminar flow cabinet (Esco).

The skin and skull were cut along the midline to the nose, and laterally to the eyes in a 'T' shape using a small pair of scissors (Figure 2-1A-D). Using forceps (Fine Science Tools, #91197-00), the skull was peeled back to expose the brain, which was carefully removed following the severing of the optic tracts and cranial nerves using a small iris spatula (Fine Science Tools, #91110-13). The brain was transferred to a dish of fresh, chilled DM. A pair of forceps with extra fine angled tips (Fine Science

Tools, #91110-10) was used to separate the midbrain and forebrain, sever the corpus callosum, and separate the hemispheres. The meninges and blood vessels were removed from the lateral surface of the hemisphere and the olfactory bulb and cerebellum were removed using a curved Iris spatula (Figure 2-1E-I). Any midbrain and meninges from the medial aspect were removed using forceps, exposing the hippocampus (Figure 2-1J). The hippocampi were inverted from the cortex and removed from the surrounding tissue by carefully pinching away the cortex. Any remaining cortical tissue was carefully removed from the hippocampus and transferred to a 15 mL Falcon® conical tube containing ice-cold DM (Figure 2-1K-M). For the dissociation and culture of cortical tissue, remaining midbrain tissue was removed and the cortex was cut into smaller pieces, and transferred to ice-cold DM (Figure 2-1N-Q)

2.1.2. *Dissociation and Plating of Primary Neurons*

Following dissection, the hippocampi were incubated in cysteine-activated papain (2.64 mM L-Cysteine (Sigma #168149), 3 % Papain (Sigma, #19001-73-4) in DM, NaOH was added to return to pH 7.4) for 15 min at 37 °C to initiate dissociation of the extracellular matrix. This incubation was repeated with fresh papain solution for an additional 15 min before tissue was thoroughly washed (4 x, 30 s) with ice-cold DM to arrest the protease action. Cells were then manually dissociated by trituration in warm neuronal growth media (NGM; 97 % Neurobasal A media, 2 % B27 supplement and 1 % Glutamax-100; Life Technologies), and any remaining tissue was allowed to settle during incubation on ice (3 min). The supernatant was removed and centrifuged (70 x *g*, 4 °C, 5 min) to pellet the dissociated cells, which were resuspended in warm NGM and counted using Trypan blue staining in a hemocytometer. Cells were plated at a density of 40,000 cells/cm^2 (40,000 total) on MatTek dishes for immunofluorescence, 67,500 cells/cm^2 (20,000 total) in 96-well plates, and 31,500 cells/cm^2 (300,000 total) or 63,000 cell/cm^2 (600,000 total) in 6-well plates for RNA or protein extraction, respectively. Cells were maintained at 37 °C (5 % CO_2) in a Heracell™ VIOS 160i CO_2 Incubator (ThermoFisher).

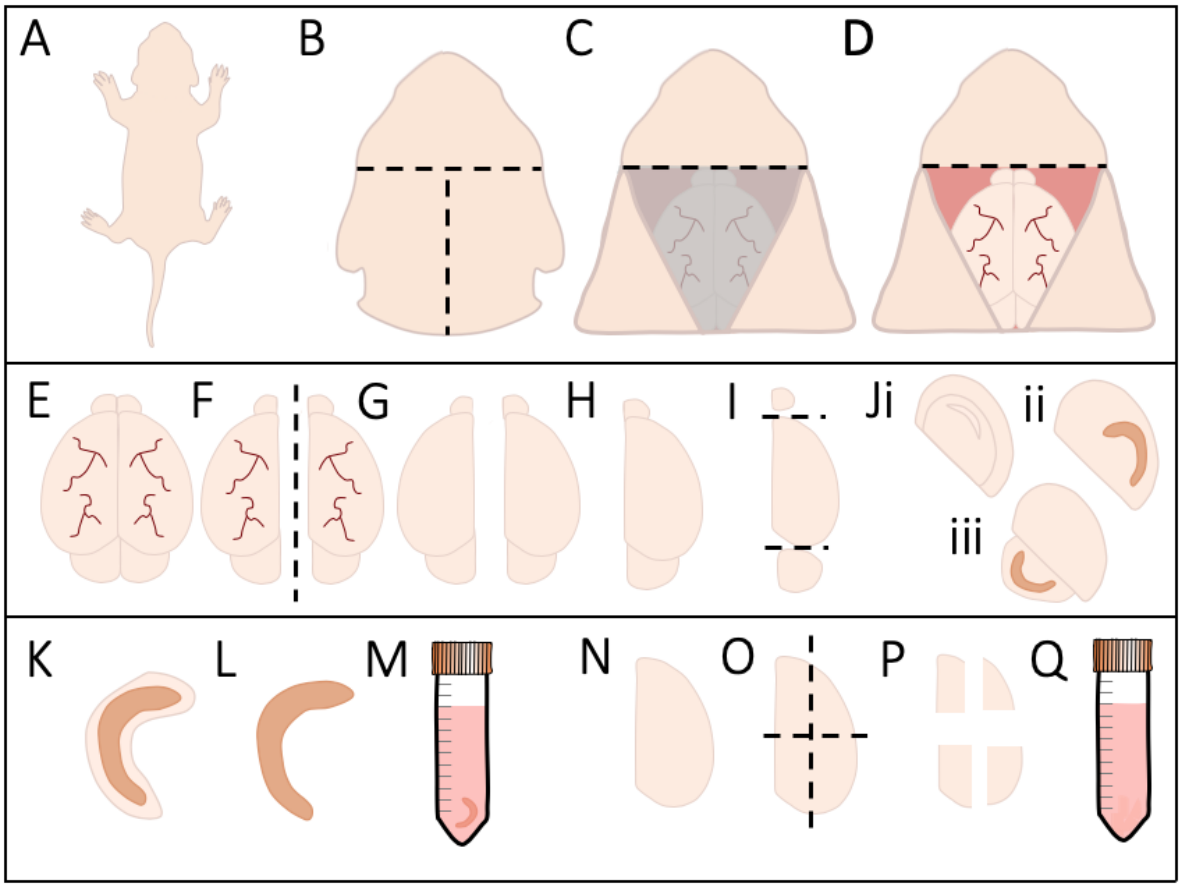


Figure 2-1 | Hippocampal and cortical dissection. A) Sprague-Dawley rat pups (PD 0-1) are decapitated. B-C) Skull is exposed by T-shaped cut down the midline, D) and the skull is removed to access the brain. E) The brain is removed and placed in ice-cold dissection media. F) The two cortical hemispheres are separated medially through the corpus callosum and G-H) the meninges are removed from the lateral surface of the cortex. I) The olfactory bulb and cerebellum are removed from each hemisphere, and Ji) the cortex is turned onto the lateral face, Jii) midbrain tissue is removed, and the hippocampus is exposed. Any remaining meninges in the hippocampal fissure and on the face of the hippocampus are removed and Jiii) the hippocampus is inverted and separated from the cortex by pinching with forceps. K-L) Any excess tissue is removed from the hippocampus and M) all hippocampi are place in 10 mL of ice-cold DM. For cortical cultures, N) remaining neocortex tissue is quartered (O-P) and Q) placed in 10 mL of ice-cold DM.

2.1.3. *Production of Conditioned Media for Cell Culture Maintenance*

Following plating, cultures were supplemented with conditioned media at four hours post-plating and 4 days *in vitro* (DIV 4). This media consisted of NGM that had been supplemented with media from healthy cultures comprised primarily of cortical neurons or glial cells, which contained secreted factors to support the growth of primary hippocampal cultures (Conde Guerri et al., 1989; Figure 2-2). For the curation of conditioned media, neuronal cells were plated in a 75 cm^2 flask coated with PDL (100 µg/mL) and maintained in NGM, with weekly feeding with NGM. Approximately 80% of the media was harvested every four days after DIV 7. For glial conditioned media, glial cells were dissociated from cortical tissue, resuspended, and plated in Minimal Essential Media (MEM; 87 % minimum essential medium (Gibco), 10 % fetal bovine serum (Gibco) and 3 % glucose (1.1 mM), supporting glial but not neuronal growth, in uncoated 175 cm^2 flasks. On DIV 1, the flask was shaken to loosen microglial attachments and remaining unhealthy neurons. The media was changed for fresh MEM to remove cell debris, and again on DIV 3 and DIV 5. On DIV 7, only glial cells remained and the MEM was replaced with NGM to support further glial cell growth and proliferation. Glial conditioned media was harvested every four days from DIV 11. Media from both flasks was harvested for up to 40 days, or until the cells appeared less healthy as determined visually by increasing debris or fragmented neuronal projections. Following collection, the media was centrifuged (69.5 x *g*, 5 min) to pellet any cellular debris and the supernatant was stored at -20 °C prior to use.

Primary hippocampal cultures were supplemented at 4 hr and 4 days post-plating with conditioned media (80 % NGM, 15 % glial media and 5 % neuronal media prepared, as described in section 2.1.3. *Production of Conditioned Media for Cell Culture Maintenance*) and maintained with weekly supplements of NGM for 21–27 DIV.

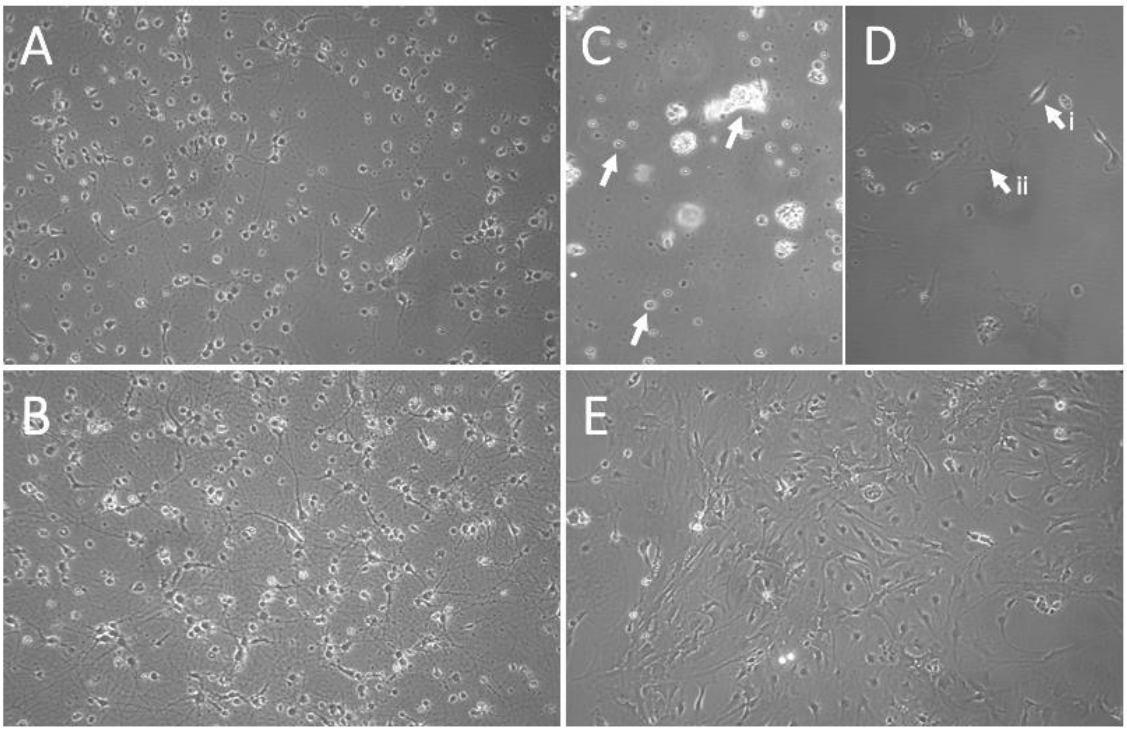


Figure 2-2 | Growth of neurons and proliferation of glia. Representative phase contrast images of A) DIV5 and B) DIV 12 neuronal flask cultures. On DIV 1 C) glial flasks show cellular debris and dead cells (arrows), removed by shaking. D) By DIV5 remaining neurons present as small cell bodies with retracting neurites (arrow, i), astrocytes begin to expand in size and multiply in number (arrow, ii). E) By DIV 12 glial cells cover most of the flask surface.

2.2. Adult Primary Neuronal Culture Preparation

2.2.1. *Dissection*

C57/Bl6 mice and C57/Bl6 mice containing the Swedish APP mutation (APP_{swe}: KM670/671NL) and PS1 exon 9 deletion (PS1Δexon9), male or female (9 months) were sourced from a breeding colony maintained at the Hercus Taieri Resource Unit by the University of Otago. Mice received an injection of pentobarbital (200 mg/kg, i.p.) before decapitation by guillotine. Brains were removed and chilled in ice-cold and oxygenated modified artificial cerebrospinal fluid (aCSF) for which sucrose was substituted for NaCl (composition in mM: sucrose 210, glucose 20, KCl 2.5, NaH_2PO_4 1.25, $NaHCO_3$ 26, $CaCl_2$ 0.5, $MgCl_2$ 3, pH 7.4 when gassed with 95% O2-5% CO2). Brains were then transferred to 100mm x 20mm petri dishes, containing ice-cold DM, and moved to a sterile laminar flow cabinet (Esco). Henceforth, adult mouse brains were obtained similar to that for primary neuronal cultures obtained from young animals (see section 2.1.1. *Dissection*). However, unlike young tissue the removal of meninges proved more difficult and therefore, for the most part, remained intact to avoid unnecessary damage to the cortical tissue underneath. Following the removal of the hippocampus, brainstem, and cerebellum, cortical tissue was diced into fine pieces and stored on ice in a 15 mL Falcon® tube containing DM for transport.

2.2.2. *Dissociation and Plating of Primary Neurons*

Dissociation and plating of cortical tissue followed a similar protocol as for young tissue (see *1.1.2. Dissociation and plating of primary neurons)*. Cells were plated at a density of 40,000-56,000 cells/cm^2 (40,000-56,000 total) on MatTek dishes. Following plating, cells were left to settle for 15 minutes to ensure attachment, before washed with warm NGM, and replaced with 1 mL conditioned media containing 10 ng/mL human basic fibroblast growth factor (bFGF; Thermofisher, #13256029). On DIV 4 cultures were supplemented with 1 mL NGM containing 10 ng/mL bFGF. Henceforth cells were fed weekly with NGM.

2.3.1. *Immunocytochemistry*

For a list of drugs and antibodies used in these experiments, see Table 2-1 and Table **2-2**, respectively.

2.3.1.1. *Treatment of Primary Cultures*

Recombinant human sAPPα and sAPPβ were produced as per Turner et al., (2009) by Dr. Megan Elder in the Tate lab. In brief, sAPPα and sAPPβ constructs were stably integrated into cultured HEK 293T cells (American Type Culture Collection). Both sAPPα and sAPPβ were secreted and purified from the culture media, and purification of proteins were assessed by a single band on an SDS polyacrylamide gel as analysed by Coomassie Blue staining, and by Western analysis. This preparation has been validated in a variety of studies *in vivo* and *in vitro* including electrophysiological and behavioural investigations (Turner et al., 2007; Taylor et al., 2008; Ryan et al., 2013; Mockett et al., 2019). Both sAPPα and sAPPβ were diluted into stock solutions in 0.1x PBS, and diluted in culture media or artificial cerebrospinal fluid (aCSF) for the final working concentration.

For experiments examining the effect of sAPPα or sAPPβ on the expression of targets of interest, cells were incubated in 0.1-1 nM sAPPα diluted in existing media for the length of treatment. Following this period, the culture media was aspirated and cells were fixed in 4 % paraformaldehyde (PFA) in phosphate buffered saline (PBS; 1.37 M NaCl, 27 mM KCl, 43 mM $Na_2HPO_4 \bullet 7H_2O$, 14 mM KH_2PO_4) containing $MgCl_2$ (1 mM), $CaCl_2$ (0.1 mM), and sucrose (PBS-MCS; 155.42 mM), for 20 min at room temperature (RT). Here, the inclusion of sucrose aides in maintaining an isotonic solution, preserving the cell membrane during fixation (Cheng et al., 2019), as well as maintaining structural integrity of internal organelles (Kisselev and Goldberg, 2005; Danchenko et al., 2019).

Table 2-1 | Catalogue of inhibitors and antagonists used throughout immunocytochemical and immunohistochemical experiments.

Drugs (Inhibitors)	*Target*	*Concentration (Stock, mM; working, µM)*	*Pre-Incubation (min)*	*Details*
Anisomycin	Translation	50; 40	30	Sigma, #A9789
Actinomycin-D	Transcription	10; 40	15	Tocris, #1229
KN62	CaMKII	5; 10	15	Tocris, #1277
PD98059	MAPK	50; 50	0	Tocris, #1213
KT5823	PKG	5; 10	0	Tocris, #1289
Chelerythrine Chloride	PKC	10; 1	30	Tocris, #1330
H-89 Dihydrochloride	PKA	2; 10	30	Calbiochem, #371963
Rapamycin	mTOR	0.86; 0.001	30	Tocris, # 1292
Sodium Fluoride	Serine/Threonine phosphatases	0.5; 1000	15	Sigma #S7920
Phenylmethylsulfonyl Fluoride	Serine/Threonine phosphatases	10; 100	15	Sigma, #P-7626
Okadaic Acid	Serine/Threonine phosphatases	10; 1	15	Tocris, #1136
Drugs (Antagonists)	*Target*	*Concentration (Stock, mM; working, µM)*	*Pre-Incubation (min)*	*Details*
α-Bungarotoxin	α7nAChR	0.01; 0.001	30	Abcam, #AB120542
IEM-1460	GluA2-lacking AMPAR	100; 100	50	Abcam, #AB141507
D(−)-2-Amino-5-Phosphonopentanoic Acid	NMDAR	50; 50	30	Sigma, #A8054
CPG 55845 Hydrochloride	$GABA_B$	25; 50	30	Tocris, #1248
ANA-12	TrkB	10; 100	30	Tocris, #4781
± A-Methyl-4-Carboxyphenylglycine	$mGluR_{II/III}$	25; 500	30	Sigma, #M-4796

2.3.2. *Primary Antibody Probe*

To examine whole cell (total) expression of a protein of interest, cells were permeabilized using 0.5% Triton X-100 in PBS (15 min); this step was omitted prior to probing only the surface-bound fraction of a target protein. In order to prevent nonspecific binding to target proteins, blocking buffer was applied to cells in preparation for probing with primary antibodies (4 % normal goat serum in PBS; NGS), for 1-hour at RT or overnight at 4 °C. Antibodies were diluted in blocking buffer and applied for

1.5 hours (FUNCAT-PLA, BioPLAy, PLA) or 2 hours (Immunocytochemistry, Immunohistochemistry, 4. *Antibody Specificity*) at RT or overnight at 4 °C. Unbound and excess antibody was removed by washing the cells in PBS (3 x 5 min).

2.3.3. *Secondary antibody probe*

Primary antibodies were detected using corresponding fluorescent-labelled secondary antibodies diluted in blocking buffer (30 min, RT). The nuclear stain DAPI was routinely included in the secondary antibody incubation (1 µg/µL) unless otherwise stated. Unbound antibody was washed from the cells with PBS (3 x 5 min), and cells were imaged immediately in PBS (pH 7.4).

Table 2-2 | Catalogue of primary and secondary antibodies used in immunocytochemistry (IC), immunohistochemistry (IH), western blot (WB), FUNCAT-PLA (F-PLA), BioPLAy (B), and PLA (PLA).

1° *Antigen*	*Host species*	*Dilution*	*Use*	*Details*
αTubulin	Mouse	1:10000	WB	Abcam, AB7291
Arc	Rabbit	1:1000	IC, IH, WB	Synaptic Systems, 156003
Biotin	Mouse	1:1000	F-PLA, B	Sigma, B7653
Biotin	Rabbit	1:1000	IC	Abcam, AB53494
GFAP	Mouse	1:1000	IC	Abcam, AB10062
GluA1 (c-terminal)	Rabbit	1:1000	IC, F, B, F-PLA, WB	Abcam, AB31232
GluA1 (N-terminal)	Mouse	1:250	IC, WB, PLA	Merk Millipore, MAB2263
GluA2 (C-terminal)	Rabbit	1:500	I, F-PLA	Merk Millipore, AB1768-I
GluA2 (N-terminal)	Mouse	1:500	IC, B, F-PLA, PLA	Abcam, 133477
GluA2 (N-terminal)	Rabbit	1:500	IC, B, F-PLA, PLA	Thermofisher, 32-0300
GluA3 (N-terminal)	Mouse	1:500	IC, B, F-PLA, PLA	Thermofisher, 32-0400
MAP2	Guinea Pig	1:1000; 1:500	IC, IH, B, F-PLA, PLA	Synaptic Systems, 188004; Abcam, AB11267
pCREB (Ser133)	Rabbit	1:500	IH	Cell Signalling, 9198
Prox1	Mouse	1:1000	IC	Abcam, AB33219
Synapsin-1	Mouse	1:1000	ICC	Synaptic Systems, 106011
Tau	Rabbit	1:500	I	Sigma-Aldrich, SAB5500182
2° *Fluorophore*	*Host Species*	*Dilution*	*Use*	*Details*
Alexa fluor 405	Goat anti-mouse	1:1000	IC	Invitrogen, A31553
Alexa fluor 488	Goat anti-mouse	1:500	IC	Thermofisher, A11001
Alexa fluor 488	Goat anti-Guinea Pig	1:1000	IC, IH, F-PLA, B, PLA	Thermofisher, A11073
Alexa fluor 488	Goat anti-rabbit	1:1000	IC	Invitrogen, A11034
Alexa fluor 555	Goat anti-mouse	1:1000	IC	Invitrogen, A21424
Alexa fluor 555	Goat anti-rabbit	1:1000	IC, IH	Invitrogen, A21429
Alexa fluor 647	Goat anti-mouse	1:500	IC	Invitrogen, A21236
IRDye680	Goat anti-rabbit	1:10000	WB	LI-COR, 926-32210
IRDye800	Goat anti-mouse	1:10000	WB	LI-COR, 926,32220
PLA^{MINUS}	Donkey anti-mouse	1:10	F-PLA, B, PLA	Sigma-Aldrich, DUO92004
PLA^{PLUS}	Donkey anti-rabbit	1:10	F-PLA, B, PLA	Sigma-Aldrich, DUO92002
Miscellaneous				
DAPI	-	1:1000 (1 µg/µL)	IC, IH, F-PLA, B, PLA	ThermoFisher, D1306
Duolink Detection Reagent Texas Red	-	1:5	F-PLA, B, PLA	Sigma-Aldrich, DUO92008

2.3.4. *Fluorescent Non-Canonical Amino Acid Tagging– Proximity Ligase Assay (FUNCAT-PLA)*

The detection of newly synthesised protein with FUNCAT-PLA was conducted in accordance with published protocol from (Dieterich et al., 2010) and adapted for current experiments. All steps carried out at RT were conducted at 21-22 °C in a temperature-controlled bench-top incubator (Benchmark Scientific, H2200-HC). Cultures used for FUNCAT-PLA experiments were between DIV21-27. The methionine analogue L-Azidohomoalanine (AHA, 4 mM; Click Chemistry Tools, #1066) was prepared in methionine-free Neurobasal A (Gibco, custom product) supplemented with 2% B27 and 1% Glutamax.

2.3.4.1. *Treatment and Incorporation of AHA*

Following visual assessment of cell health, dishes were randomly assigned to treatment groups for each experiment. Following the removal of existing media, treatment dishes received 1 nM sAPPα (diluted from 320 nM stock) in 4 mM AHA (prepared as described in section 2.3.4 *Fluorescent Non-Canonical Amino Acid Tagging– Proximity Ligase Assay (FUNCAT-PLA)*. Control groups received only AHA-containing NGM for the length of the treatment. Following the incubation period, the cultures were immediately washed three times in PBS-MC (pH 7.4, RT) then fixed in 4 % PFA in PBS-MCS (20 min, RT). Cells were incubated in blocking buffer (4 % normal goat serum in PBS, 1 hr at RT), and then washed twice in PBS pH 7.8 (2 x 10 min, RT) to slowly raise the pH in preparation for the click reaction.

2.3.4.2. *Click Reaction*

The detection of the AHA-containing, newly synthesised proteins, is made possible through addition of an alkyne-bearing biotin moiety. This moiety can be covalently attached to the azide-bearing AHA through a copper(I)-catalysed [3+2] azide-alkyne cycloaddition or 'click chemistry' (see Figure 2-3). The click reaction mixture was prepared immediately prior to application to the cells. The mixture was comprised of 200 µM Tris (1-benzyl-1H-1,2,3-triazol-4-yl)methyl) amine (TBTA triazole ligand, Aldrich), 500 µM Tris(2-carboxyethyl)phosphine hydro-chloride (TCEP, Thermo Scientific), 25 µM Biotin-PEG4-alkyne (Biotin alkyne, Aldrich) and 200 µM $CuSO_4$ in PBS pH 7.8. All reagents were dissolved sequentially through vigorous vortexing after the addition of each component from stock solutions.

Immediately following preparation, 1000 µL of 'click mix' was added to each dish, and incubated overnight at RT.

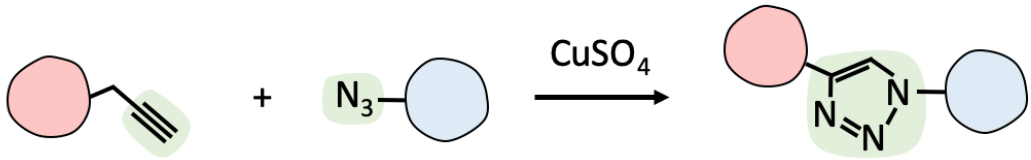


Figure 2-3 | Copper-catalysed azide-alkyne cycloaddition (CuAAC). An alkyne-bearing biotin (●) can be covalently attached to the azide-bearing AHA (●) via a Copper-catalysed click-chemistry reaction.

2.3.4.3. *Detection of Newly Synthesized Proteins*

Following the 'click'-mediated labelling of newly synthesized cell surface proteins with a biotin moiety, FUNCAT-PLA was employed to visualise their expression and location (Figure 2-4). Cells were washed with PBS (pH 7.8, 2 x 10 min), permeabilized with 0.5 % Triton X-100 in PBS (15 min, RT), and incubated in blocking buffer (1 hr, RT). Cells were then probed with primary antibodies against biotin, MAP2, and the target protein (GluA1 or GluA2; refer Table **2-2**; Appendix 4. *Antibody Specificity*), in blocking buffer (1.5 hr, RT). Following incubation, unbound antibody was removed by washing with PBS (3 x 5 min) prior to addition of proximity ligation assay (PLA) probes.

2.3.4.4. *Proximity Ligation Assay (PLA)*

Detection of newly synthesized proteins was carried out using proximity ligation assays (PLA; Figure 2-4). The probes used in PLA recognize the constant region of the primary antibody or antibodies. The maximum proximity of these antibodies for successful ligation has been cited as ≤ 16 nm (Trifilieff et al., 2011) to ≤ 40 nm (Bagchi et al., 2015). Once in proximity, the DNA strands attached to the probes participate the generation of a concatemeric 'rolling circle amplification' product, when exposed to the appropriate substrates and enzymes. A dilution (1:10) of Donkey PLA^+ and PLA^- probes (Duolink, Sigma-Aldrich; see Table **2-2**) was prepared in blocking buffer containing anti-guinea pig 488 secondary antibody (1:1000) and DAPI (1:1000), where possible. This mixture was applied to the cells (37 °C, 1 hr) in a humidified chamber; the dishes were placed on a metal tray surrounded by wet paper towels, double-sealed in two zip-lock bags. Following incubation, the cells were washed using Wash Buffer A (0.01 M Tris base, 0.15 M NaCl, 0.05 % Tween 20, pH 7.4; 4 x 5 min, RT; Duolink,

Sigma-Aldrich) prior to the addition of ligation mixture (containing circularization oligomers complementary to those conjugated to the PLA probes, and T4 ligase (1 U/µL) diluted in MQ according to manufacturer's instructions; 30 min, 37 °C; in a humidified chamber).

Binding of PLA^+ and PLA^- probes permits the ligation of the circularization oligomers, forming the basis of the rolling circle amplification process. Following this, the ligation reaction mixture was washed from the cells using Wash Buffer A (4 x 5 min, RT) prior to addition of the amplification solution (containing fluorophore-labelled (λ_{ex} 594 nm; λ_{em} 624 nm) nucleotides for the rolling circle reaction, and Phi29 polymerase (10 U/µL), diluted in MQ according to Duolink's instructions; 100 min, 37 °C, in a humidified chamber). Following amplification, cells were washed in Wash Buffer B (0.2 M Tris-HCl, 0.1 M NaCl, pH 7.5; 2 x 10 min, RT; Duolink, Sigma-Aldrich) in order to arrest the polymerase reaction. Excess Tris-HCl was washed from the cells using PBS (pH 7.4), and the cells were then post-fixed to support the stability of the PLA signal (4 % PFA in PBS, 20 min), washed (4 x 5 min, PBS, pH 7.4) and stored in PBS (4 °C). The resultant fluorescent signal is approximately 500 nm and can be effectively detected using fluorescence microscopy.

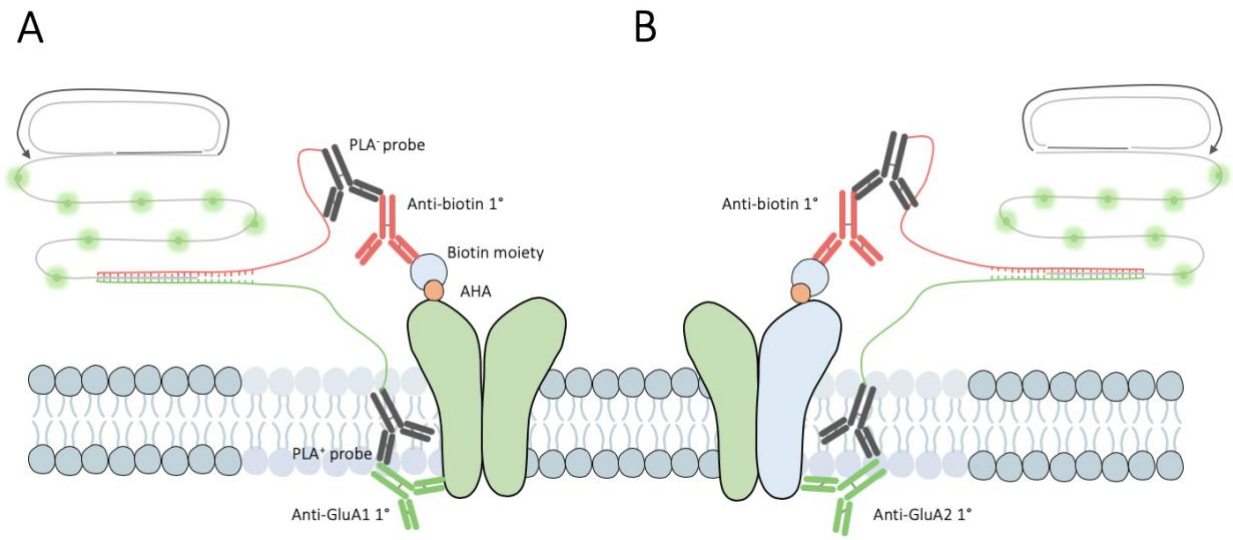


Figure 2-4 | Schematic diagram of FUNCAT-PLA experimental design. Newly synthesised cell surface A) GluA1 or B) GluA2 proteins are targeted by incubation with AHA (●) and 'click'-mediated addition of a biotin moiety (●). Following permeabilization of the plasma membrane (●), primary anti-biotin (●) and A) anti-GluA1 or B) anti-GluA2 antibodies (●) are added, followed by PLA^+ and PLA^- probes (●). Incubation of PLA probes with appropriate ligation and amplification reagents allows for formation of the rolling circle amplification and addition of fluorescent probes (●).

2.3.5. *Detection of Cell Surface Proteins Using BioPLAy*

For the detection of cell surface receptors, we employed a novel technique, which incorporates biotinylation of surface proteins with proximity ligation assays (BioPLAy; Figure 2-5). All steps carried out at RT were conducted at 21-22 °C in a temperature-controlled bench-top incubator (Benchmark Scientific, H2200-HC). All PBS used was at pH 7.4, and cultures were used for experiments between DIV21-DIV27.

2.3.5.1. *Treatment*

Following a visual assessment of cell health, dishes were randomly assigned to treatment groups for each experiment. Following the removal of existing media, treatment dishes received 1 nM sAPPα (diluted from 320 nM stock in NGM) diluted in NGM. Control groups received only NGM for the length of the treatment.

2.3.5.2. *Biotinylation*

In order to isolate the cell surface fraction of GluA1-containing AMPAR, the cell-impermeant Sulfo-NHS-SS-Biotin was applied following treatments. Immediately prior to application, EZ-Link Sulfo-NHS-SS-Biotin (2.5 mg/mL; ThermoFisher Scientific, #21331) was dissolved in PBS-MC (1 mL, RT). Culture media was aspirated from the experimental dishes and the dissolved biotin conjugate was added to each dish on ice (10 min, 4 °C), to arrest biological processes, including those involved in receptor endo- and exocytosis. Excess biotin was washed from the cells using ice-cold PBS-MC (3 x, quickly). The cells were immediately fixed in 4 % PFA in PBS-MCS (20 min, RT).

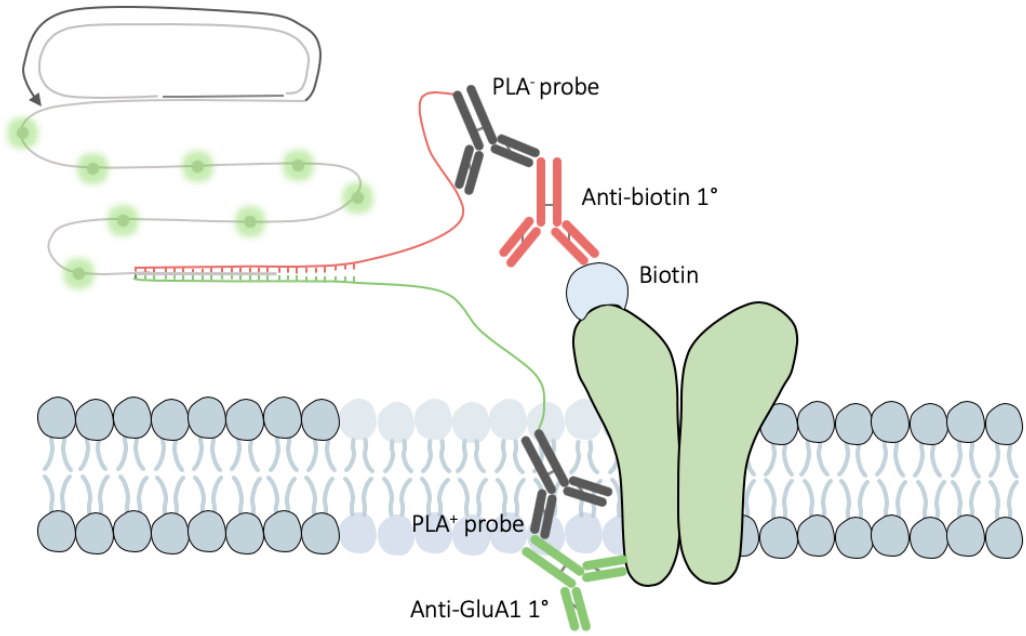


Figure 2-5 | Schematic diagram of BioPLAy experimental design. Cell surface GluA1 proteins are targeted by addition of Sulfo-NHS-SS-Biotin (●). Following permeabilization of the plasma membrane (●) primary anti-biotin (●) and anti-GluA1 (●) antibodies are added, followed by PLA⁺ and PLA⁻ probes (●). Incubation of PLA probes with appropriate ligation and amplification reagents allows for formation of the rolling circle amplification and addition of fluorescent probes (●).

2.3.5.3. *Proximity Ligation Assay (PLA)*

Proximity ligation assay was performed as described in section 2.3.4.4. *Proximity Ligation Assay (PLA)*. Briefly, the cells were permeabilized in 0.5 % Triton X-100 in PBS (15 min, RT), and incubated in blocking buffer (4 % normal goat serum in PBS, 1 hr at RT, or overnight at 4 °C). Following this, all dishes were incubated with the primary antibodies of interest (1.5 hr, RT; refer Table **2-2**). Cells were then washed (PBS pH 7.4, 3 x 5 min) and incubated in PLA⁺ and PLA⁻ probes (1:10 in blocking buffer) with the addition of Alexafluor 488-bound goat anti-Guinea pig secondary antibody (1:1000) and DAPI (1 µg/µL; 1 hr, 37 °C, in a humidified chamber). Unbound probes were washed with Wash Buffer A (4 x 5 min, RT). Dishes were incubated with the ligation mixture (30 min, 37 °C, in a humidified chamber), and washed with Wash Buffer A (4 x 5 min, RT) prior to incubation with the amplification reaction mixture (100 min, 37 °C, in a humidified chamber). Cells were then washed in Wash Buffer B (2 x 10 min, RT) The cells were finally washed (3 x 5 min) and imaged immediately in PBS (pH 7.4).

2.3.6. *Detection of Cell Surface Receptor Subunit Dimers (PLA)*

Similar to BioPLAy (see section 2.3.6. *Detection of Cell Surface Proteins Using BioPLAy*), PLA-only implements surface labelling of AMPA receptor subunits with epitope-specific antibodies to allow for formation of 'rolling circle amplification' product upon addition of appropriate PLA probes.

2.3.7. *Treatment*

Treatment of experimental cultures was carried out as described in section 2.3.5.1. *Treatment*. In brief, treatment dishes received 1 nM sAPPα (diluted from 320 nM stock), in NGM following aspiration of existing media from the dishes. Control groups received NGM-only for the length of the treatment.

2.3.8. *Addition of Antibodies*

Immediately prior to application, cells were washed (PBS-MC; 3 x, quickly) and immediately fixed in 4% PFA in PBS-MCS (20 min, RT). For experiments investigating GluA1/2-containing AMPAR (Figure 2-6A), using both C-terminal and N-terminal antibodies, cells were incubated in blocking buffer (4 % normal goat serum in PBS, 1 hr at RT), probed with the N-terminal antibody (1.5 hr, RT), washed (PBS pH 7.4, 3 x 5 min), and subsequently permeabilized with 0.5 % Triton X-100 in PBS (15 min, RT). Cells were then probed with the C-terminal antibody in addition to guinea pig anti-MAP2 for visualization of neuronal structure (1.5 hr, RT) and washed (PBS pH 7.4, 3 x 5 min).

For experiments utilizing two N-terminal antibodies, such as those investigating GluA2/3-containing AMPAR (Figure 2-6B), permeabilization was omitted and cells were incubated in blocking buffer (4 % normal goat serum in PBS, 1 hr at RT) followed by incubation of primary antibodies (1.5 hr, RT). Following this, cells were washed (PBS, pH 7.4; 3 x 5 min), permeabilized (0.5 % Triton X-100 in PBS, RT; 15 min) and incubated in blocking buffer again (4 % normal goat serum in PBS, 1 hr at RT) before addition of anti-MAP2 for visualization of neuronal structure (1.5 hr, RT).

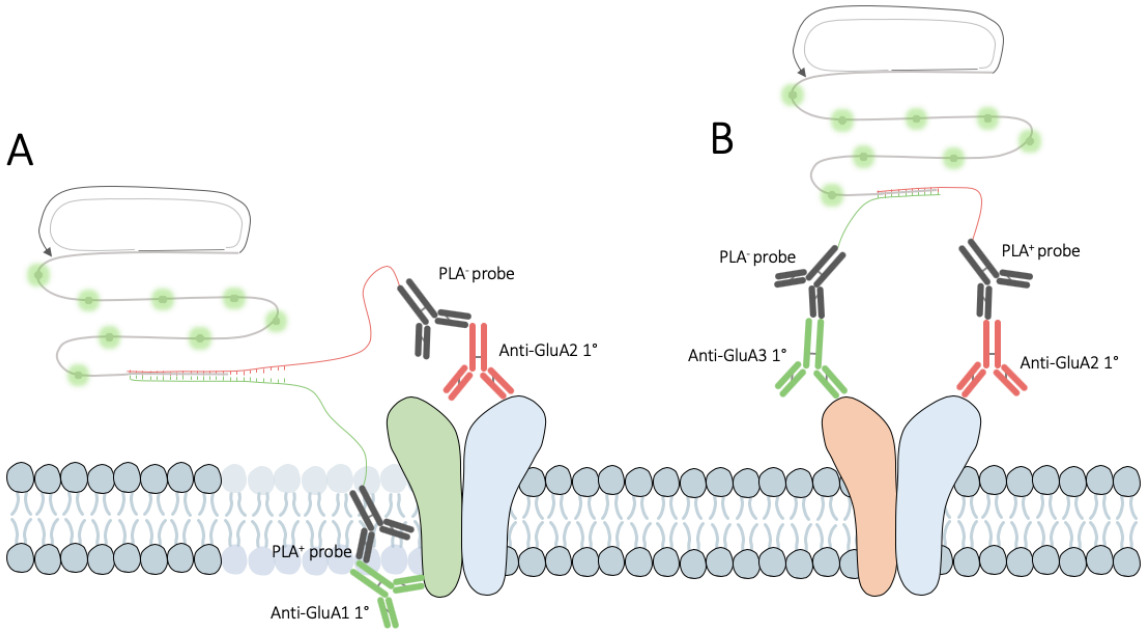


Figure 2-6 | Schematic diagram of A) GluA1/2 and B) GluA2/3 PLA experimental design. A) Cell surface GluA1/2 AMPAR were targeted by addition of N-terminal GluA2 antibody (●) before permeabilization of the plasma membrane (●). Following permeabilization C-terminal primary anti-GluA1 (●) are added. B) Cell surface GluA2/3 AMPAR were targeted by addition of N-terminal GluA2 (●) and N-terminal GluA3 (●) antibodies, before permeabilization. Both GluA1/2 and GluA2/3 AMPAR complexes were targeted by application of PLA⁺ and PLA⁻ probes (●). Incubation of PLA probes with appropriate ligation and amplification reagents allows for formation of the rolling circle amplification and addition of fluorescent probes (●).

2.3.9. *Proximity Ligation Assay*

Proximity ligation assay was performed as previously described in sections 2.3.4.4. *Proximity Ligation Assay (PLA)* and 2.3.5.3. *Proximity Ligation Assay (PLA)*. In brief, cells were incubated in PLA⁺ and PLA⁻ probes (1:10 in blocking buffer) with the addition of Alexafluor 488-bound goat anti-Guinea pig secondary antibody and DAPI (1:1000; 1 hr, 37 °C; humidified chamber). Unbound probes were washed with Wash Buffer A (4 x 5 min, RT). Dishes were incubated with the ligation mixture (30 min, 37 °C; in a humidified chamber), and washed with Wash Buffer A (4 x 5 min, RT) prior to incubation with the amplification reaction mixture (100 min, 37 °C, in a humidified chamber). Cells were then washed in Wash Buffer B (2 x 10 min, RT) and probed with DAPI (1 µg/µL, 10 min). The cells were finally washed (3 x 5 min) and imaged immediately in PBS (pH 7.4).

2.3.10. *Treatment of Cultures With siRNA*

To investigate the effect of siRNA knockdown of Arc protein following sAPPα treatment, we employed treatment of cultures with Accell™ Arc siRNA (1 µM) targeted to the open reading frame (ORF) of the Arc gene (see Table 2-3). Cells were pre-treated with Arc siRNA for 1 hour before co-incubation ± sAPPα (1 nM, 2 hr) and AHA (4 mM). Cells were processed for FUNCAT-PLA (see section 2.3.4. *Fluorescent Non-Canonical Amino Acid Tagging– Proximity Ligase Assay (FUNCAT-PLA)* or Immunocytochemistry (see section 2.3.1. *Immunocytochemistry)*. Alongside this, the non-targeting (NT) control siRNA was used to assess effects, if any, of the addition of small molecules in the interpretation of results. Additionally, to assess efficacy of Accell™ siRNA expression in our cultures we employed the Accell™ Red non-targeting control siRNA (1 µM; Table 2-3). This siRNA contains a fluorophore conjugated to a non-targeting sequence, able to be visualized by conventional epifluorescence microscopy. Cultures were pre-treated for 1 hour, followed by an additional 2 hours in line with experimental treatment lengths. Cells were processed for immunocytochemistry for markers of cell structure (MAP2, DAPI) under dark conditions to maintain fluorescence signal.

All Accell™ siRNA were prepared in a 1x siRNA reconstitution buffer consisting of (in mM): KCL 300, $MgCl_2$ 1, HEPES 30, and pH'd to 7.3-7.6 by addition of 2M KOH. All reagents were prepared in RNAse-free water and sterile filtered. siRNA were reconstituted in 1x siRNA reconstitution buffer and incubated at 37 °C with gentle rocking for 70 min. Reconstituted siRNA were aliquoted and stored at -20°C until needed. For treatment of cultures, siRNA were further diluted to a working concentration of 1 µM in NGM ± AHA alongside treatment conditions.

Table 2-3 | Summary table of siRNA and their targets used in FUNCAT-PLA experiments.

Target	*Target Sequence*	*Molecular Weight (g/mol)*)	*Extinction Coefficient ($L/mol^{-1}cm^{-1}$)*	*Details*
ARC	CUGCAGUACAGUGAGGGUA	13,511.8	346,388	Dharmacon, A-080172-15-0020
NON-TARGETING	UGGUUUACAUGUGUCGACUAA	13,400	150,000	Dharmacon, D-001910-01-20
Target	*Target Sequence*	*Fluorophore*	*Absorption/ Emission*	*Details*
RED NON-TARGETING	UGGUUUACAUGUGUCGACUAA	DY-547	557/570 nm	Dharmacon, D-001960-01-05

2.4. Reverse Transcriptase Quantitative Polymerase Chain Reaction (RT-qPCR)

2.4.1. *RNA Isolation and Purification*

For the quantification of mRNA from culture, cells were treated as per Section 2.3.1.1. *Treatment of Primary Cultures*. Total RNA was extracted from cortical cell cultures prepared in 6-well plates at a density of 31,500 cells/cm^2. Tissue was lysed by using 350 µl Buffer RL (Norgen Biotek Corp., #17200) directly in the culture plate and gentle swirling for 5 min at RT. Tissue was transferred to an RNase-free microcentrifuge tube to which 200 µl ethanol was added. RNA was bound to RNA-binding spin columns (Norgen Biotek Corp., #17200) by centrifugation for 1 min at 3,500 x *g*. This was followed by an on-column DNA removal, by passing 400 µl of Wash Solution A through the column by centrifugation (1 min, RT, 14,000 x *g*), followed by 100 µl RNase-free DNase solution, centrifugation (1 min, RT, 13,000 x *g*). This was repeated three times before incubation (15 min, 25–30°C) and centrifugation for a final time (2 min, RT, 13,000 x *g*) to thoroughly dry the resin. Purified RNA was eluted by addition of Elution Solution A (50 µl; Norgen Biotek Corp., #17200) to the column, centrifugation (2 min, RT, 200 x *g,* followed by 1 min, 13,000 x *g*) and collection in an RNase-free microcentrifuge tube kept on ice. A second elution of 50 µl was performed as above into a separate collection tube.

2.4.1.1. *RNA Quality and Quantification*

Using a Nanodrop ND-1000 v3.8.1 Spectrophotometer and associated software (Thermofisher Scientific), 2 µl of RNase-free water was pipetted onto the measurement pedestal and used as a blank measure. Following this, 2 µl of RNA sample was pipetted onto the pedestal, measured, and the pedestal cleaned. This was repeated for both primary and secondary elution separately. The quality of each of the RNA samples can be determined primarily by the A260/A280 ratio, wherein a ratio of approximately 1.8–2.0 indicates a 'pure' RNA sample, free of contaminants such as proteins, salts, or phenol. Following a 'pure' reading from each of the elution samples, both primary and secondary elution were combined and a final quality and concentration report was generated using the Nanodrop system, resulting in approximately 100 µl of 'pure' RNA.

2.4.1.2. *Primer Design*

For the quantification of RNA following treatment, the following primers were designed using Primer-BLAST (https://www.ncbi.nlm.nih.gov/tools/primer-blast/; see Table **2-4**), under the conditions that the primer must span an exon-exon junction and was specific for rat. Primers were hence selected based on specific amplification of the gene of interest, the T_m of each primer were within 5°C of each other, and the percentage of guanine (G) or cytosine (C) present was between 40% and 60%. Candidate primers were also checked for primer dimerization (binding with itself), repeats (nucleotide sequences repeating), and hairpin binding, features which may affect primer efficiency during the RT-qPCR reaction.

Table 2-4 | Summary table of primer sequence pairs used in RT-qPCR experiments.

Gene Target	*Forward Primer*	*TM (°C)*	*Reverse Primer*	*TM (°C)*
ZIF268	5'-GGG AGC CGA GGG AAC AA-3'	58.2	5'-CGT TAT TCA GAG CGA TGT CAG AA-3'	54.8
HPRT	5'-TGA CAC TGG TAA AAC AAT GCA-3'	52.8	5'-GGG AGC CGA GCG AAC AA-3'	58.2
ARC #1	5'-AGC AGA ATC AGA GAT GGC CG-3'	57.2	5'-TGA ATC ACT GCT GGG GGC-3'	58.0
ARC #2	5'-GAC TTG ATT GAG CTG GGG CT-3'	57.5	5'-TAT GAA TCA CTG CTG GGG GC-3'	57.3

2.4.1.3. *cDNA Synthesis*

cDNA libraries were created from the total RNA extracted from cultured cortical neurons (*n* = 1-4 wells per condition, 4-5 experimental replicates) using SuperScript III RT (Invitrogen). Following the manufacturer's instructions for a 20 µl reaction volume, Master Mix I was made by adding 1 µl Oligo$(dT)_{20}$ (50 µM) and 1 µl dNTP mix (10 mM) to a 0.6 mL RNase-free microcentrifuge tube. Master Mix II contained 4 µl 5X First Strand Buffer, 1 µl dithiothreitol (DTT; 0.1 mM), 1 µl RNaseOUT (40 U/µl), and 1 µl SuperScript III RT (200 U/µl). Following this, 5 ng of total RNA was added to an RNase-free tube followed by Nuclease-free H_2O for a total volume of 11 µl. This was then heated at 65°C for 5 min via thermocycler, and incubated on ice for 1 min. 7 µl of Master Mix II was then added and mixed via pipette. cDNA samples were stored at -20 °C prior to use.

2.4.1.4. *SYBR Green mRNA Assays*

To test the efficiency of each primer, 5 µl of cDNA from each test sample was combined and serially diluted with RNase-free H_2O at 1:1, 1:5, 1:25, and 1:125. Following qPCR amplification and data acquisition, the triplicates were averaged and concentrations were converted to $\log_{10}$ values and graphed onto a scatter plot. A line of best fit, as determined by Excel 2011 (Microsoft), was generated giving an m and an R^2 value. A primer set was determined to be efficient using the equation $E = 10^{-1/m}$ x 100, and with an R^2 value greater than 0.90. In certain cases, the highest or lowest value was removed as they may affect the line of best fit, due to PCR inhibition or low concentrations of RNA, respectively. Primers were also deemed efficient by the melting curves generated following the PCR to identify a specific amplification is occurring (Figure 2-7). The presence of a singular peak indicated a single RNA product had been amplified while a double peak indicated nonspecific amplification or primer dimers. Due to this, Arc primer #2 was not used for any experiments.

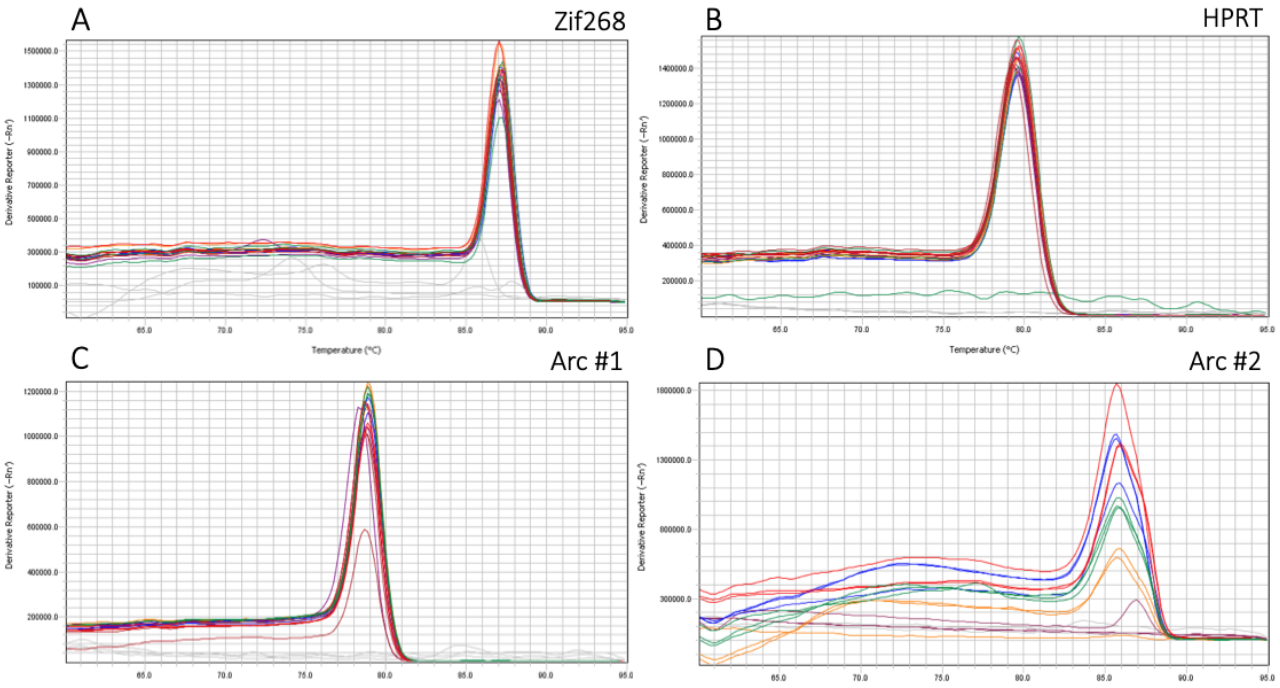


Figure 2-7 | Melt curve plots. Graphs show A) Zif268, B) HPRT, C) Arc #1, D) Arc #2, E) Gria1, F) Gria2, and G) Gria3. Axes show the derivative reporter (−Rn′) plotted against temperature (°C).

2.4.2. *Reverse-Transcriptase Quantitative Polymerase Chain Reaction (RT-qPCR)*

Each primer was tested at a 1:25 dilution and each condition was tested in triplicate. On a 96-well plate (Roche, #0472962001), each well contained 3 µl cDNA and 7 µl of a premade master mix consisting of 1 µl Forward Primer (5 µM), 1 µl Reverse Primer (5 µM) and 5 µl SYBR Green Master Mix (ThermoFisher Scientific, #4309155). Using a LightCycler 480 and its associated software, setting the sample volume to 10 µl, each plate underwent a pre-incubation program at 95°C for 5 min. Following this, an amplification program with a quantification analysis involved 40 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 30 s. Following amplification, a melting curve analysis was carried out with 95°C for 15 s, 60°C for 1 min, followed by continuous ramp at 0.05 °C/s up to 95°C before cooling to 40°C and holding for 30 s (Figure 2-8)

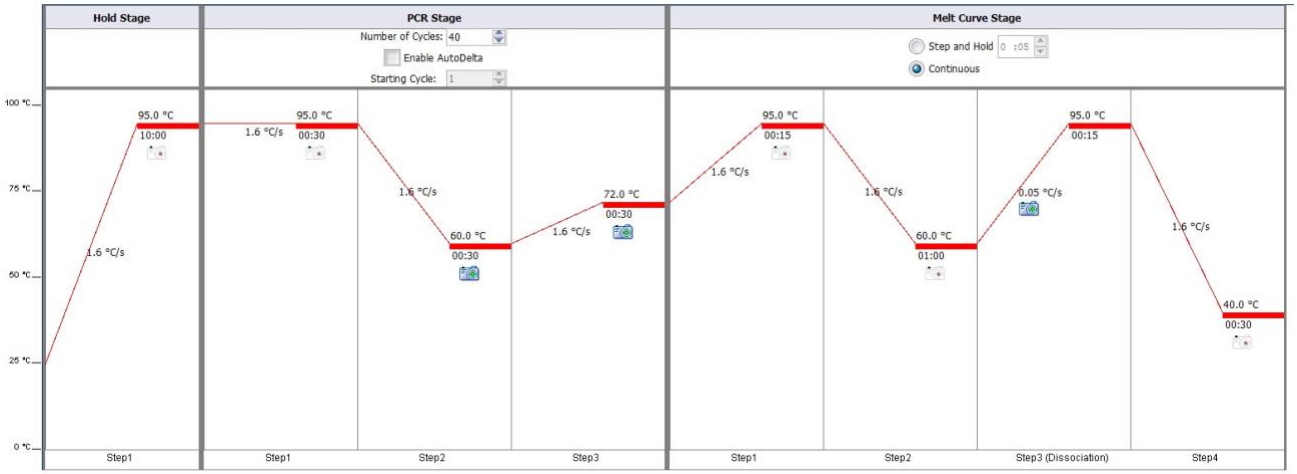


Figure 2-8 | Experimental setup of RT-qPCR amplification and quantification cycles. Image shows progress of RT-qPCR experiments during hold stage, PCR, stage, and melt curve stage. Red line indicates rate of degree change (°C/s), holding temperature (°C) and holding duration (min).

2.4.3. *Western Blot*

Western blotting was utilised to validate the binding of antibodies to a target of the expected size as the proteins of interest, to be used in immunocytochemical- and PLA-based experiments.

2.4.3.1. *Protein Extraction and Sample Preparation*

Primary hippocampal cultures were grown for protein harvesting on Poly-D-lysine coated wells of a 6-well plate, and harvested at DIV24-27. The cell layer was washed (PBS-MC, pH 7.4), scraped from the well and collected by centrifugation (14,000 x *g*, 5 min, 4 °C). The pellet was washed with PBS and centrifuged again before the cells were lysed and homogenised in buffer (1 mM EGTA, 1 mM EDTA, 0.1 mM PMSF, 1 x Complete, 1 % Triton-X, 10 % SDS, 10 µM KN62 in PBS). The samples were sonicated (Bandelin Sonorex Digital Ultrasonic bath; 2 min, RT), and centrifuged (20 min, 14 000 x *g*, 4 °C). The supernatant was collected, and the pellet was re-homogenised. Protein was quantified using the BCA assay and stored at - 80 °C until use.

2.4.3.2. *Gel Electrophoresis and Transfer*

SDS-Polyacrylamide resolving gels (9% Bis-Acrylamide (Bio-Rad), 0.5 mM SDS, 0.05% TEMED, 0.05% APS buffered with 0.37 M Tris-HCl) were cast in NOVEX 1 mm gel cassettes (ThermoFisher). The gels were sealed with water overnight at 4 °C. Stacking gels (5% acrylamide, 0.5 mM SDS, 0.1% TEMED, 0.05% APS buffered with 0.125 M Tris-HCl) were cast, and the gels were left at RT for 20 min. NOVEX cassettes were locked into a NOVEX X-cell Surelock Minicell system (Invitrogen). Inner and outer chambers of the box were filled with sufficient running buffer (192 mM glycine, 0.1% SDS buffered with 25 mM Tris-HCl) to cover the wells in the gels. The prepared samples were pipetted into the wells alongside a molecular weight rainbow marker (Amersham, GE Healthcare Life Sciences). Electrophoresis was conducted at 125 V for 2 hr, or until the dye front reached the base of the gel. The separated proteins were then transferred to nitrocellulose membranes (Amersham Protran 0.45 µm NC, GE Healthcare Life Science) using NOVEX X-cell II blotting modules (Invitrogen). The transfer was carried out at 100 mAmp for 2 hr, using transfer buffer (96 mM glycine, 10 % methanol buffered with 12 mM Tris-HCl).

Following transfer, blocking buffer was applied to the membranes (Odyssey blocking buffer; Li-Cor; 1 hr, RT). Primary antibodies were diluted in 0.1 % BSA, 0.1 % NGS in PBS with 0.1% Tween, and membranes were probed overnight at 4 °C with gentle rocking. The following day, unbound antibody was removed using PBS-Tween (4 x 5 min) and the membranes were incubated with the appropriate secondary antibodies in PBS-Tween (1 hr, RT, gentle rocking). Unbound antibody was washed using PBS-Tween (4 x 5 min) and the membranes were washed in PBS, before being allowed to dry on filter paper prior to scanning. Protein was detected using the LI-COR Odyssey Infrared Fluorescence Imaging system. Image capture was carried out using Image Studio. The brightness and contrast levels of captured images were adjusted for image presentation.

2.5. Preparation of Acute Hippocampal Slices for Electrophysiology and Biochemical Experiments

All experimental protocols were approved by the University of Otago Animals Ethics Committee and conducted in accordance with New Zealand Animal Welfare Legislation under the ethics approval DET19/16. All experiments conducted on acute tissue were prepared from young adult male Sprague-Dawley rats (4-6 weeks), as described previously (Mockett et al., 2019).

2.5.1. *Acute Hippocampal Slice Preparation*

Rats were deeply anaesthetised with ketamine (100 mg/kg, i.p.) and decapitated by guillotine. The brains were removed and chilled in ice-cold and oxygenated modified artificial cerebrospinal fluid (aCSF) for which sucrose was substituted for NaCl (composition in mM: sucrose 210, glucose 20, KCl 2.5, NaH_2PO_4 1.25, $NaHCO_3$ 26, $CaCl_2$ 0.5, $MgCl_2$ 3, pH 7.4 when gassed with 95% O2-5% CO2). Following removal, the brain was transferred to an ice-cold dissection platform (Figure 2-9A,B). Hemispheres were separated and cerebellum were removed. Ice-cold aCSF was applied throughout to ensure the tissue remained cold and rigid. Using a razor blade, a cut through the forebrain (at the point of the middle cerebral artery) was made, and the hemisphere was flipped onto the cut face, exposing the midbrain (Figure 2-9C-E). Midbrain tissue was removed using a flat spatula, revealing the medial surface of the hippocampus. The tissue was rolled onto the lateral surface of the cortex. The flat spatula was then used to slide beneath the hippocampus, carefully flipping it out above the cortical tissue, lateral surface facing up (Figure 2-9F-H). The hippocampus was separated from the cortex and two 45° cuts were made at each end, loosening the hippocampus from remaining cortical tissue (Figure 2-9I). From here the ventral portion of the hippocampus was removed. Area CA3 was removed by removal of the lower $1/10^{th}$ of the remaining dorsal hippocampus, in order to increase the signal-to-noise ratio of recordings by reducing the amount of spontaneous activity (Dumas et al., 2018; Figure 2-9J,K). From here, the hippocampal tissue was glued gently to the vibratome mounting platform with the lateral surface facing out and the dorsal surface facing up. Hippocampi were dissected at a thickness of 400 µm, using a vibroslicer (Leica, VT1000; Figure 2-9L-N). Slices were transferred to a porous, transparent membrane in an incubation chamber, and maintained at the interface between air and standard aCSF (in mM: NaCl 124, KCl 3.2, NaH2PO4 1.25, NaHCO3 26, CaCl2 2.5, MgCl2 1.3, D-glucose 10, equilibrated with carbogen 95% O2-5% CO2; 32°C). Slices remained here for 30 minutes before transfer to RT for an additional 90 minutes (Edwards and Konnerth, 1992; Lein et al., 2011).

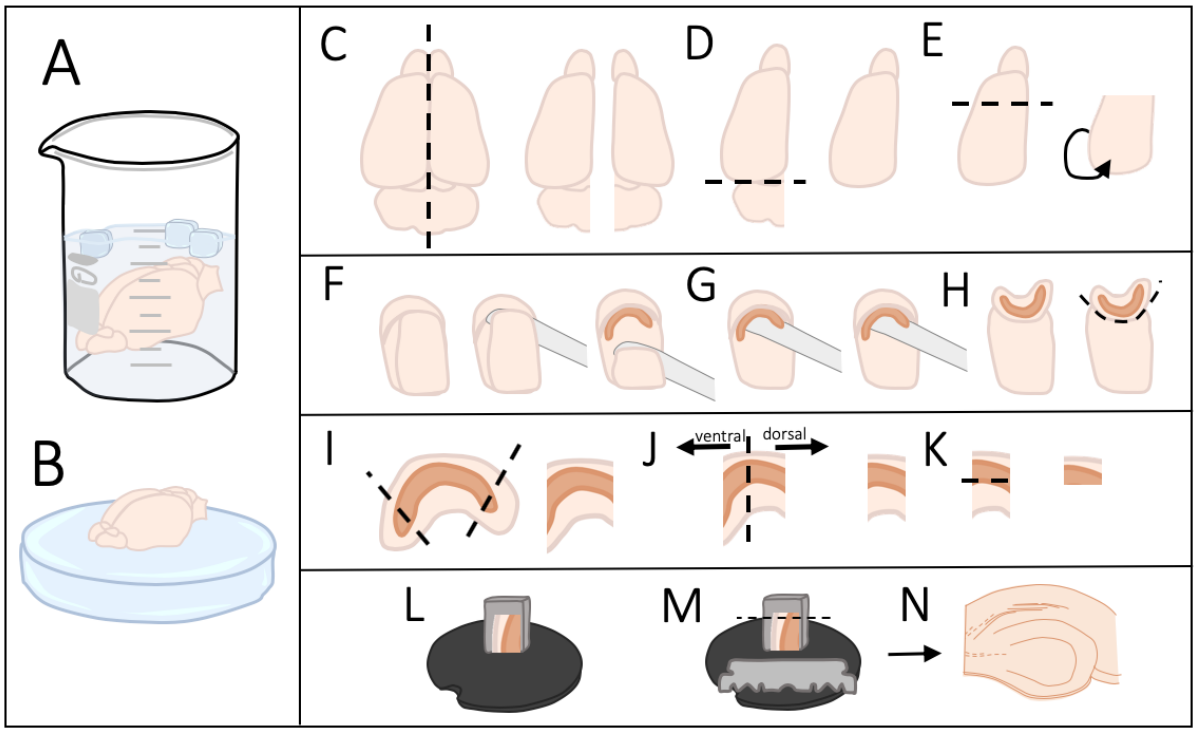


Figure 2-9 | Acute transverse hippocampal slice dissection. Following decapitation and removal of the brain, A) brains are transported in oxygenated ice cold modified aCSF and transferred to B) a chilled dissection platform. C) Hemispheres are separated and D) the cerebellum is removed. E) The forebrain is removed by a vertical slice at the point of the middle cerebral artery. Remaining hemisphere is flipped onto the cut face. F) Midbrain tissue is removed, revealing the hippocampus. G) Using the spatula, the hippocampus is carefully flipped and H) separated from the remaining cortex. I) Additional tissue at the end of the hippocampus are removed by two 45° cuts, J) followed by removal of the most ventral aspect. K) The CA3 area is removed from the remaining dorsal tissue by vertical slice of approximately the lower 1/10th of the remaining tissue. L) The ventral surface is glued to the vibratome mounting platform M-N) and 400 µm transverse sections are cut.

2.5.2. *Immunohistochemistry*

sAPPα and other drug treatments were subsequently applied in warmed aCSF for 15 minutes or 2 hours. When studying inhibitor effects on sAPPα treatment, slices were pre-incubated for 30 minutes with the inhibitors (αBGT, 10 nM; APV, 50 µM) before subsequent co-incubation with sAPPα for 2 hours. When studying the effects of sAPPα treatment on phosphorylated CREB (pCREB) expression, slices were co-incubated with inhibitors of serine/threonine-protein phosphatases (Sodium Fluoride (NaF), 1 mM; Phenylmethylsulfonyl Fluoride (PMSF), 100 µM; Okadaic acid, 1 µM). Following treatment, slices were washed in PBS-MC (pH 7.4) and subsequently fixed in 4% PFA in PBS-MCS (pH 7.4) overnight at 4°C. Following fixation, slices were washed in PBS (pH 7.4) and embedded in Agarose (3 %; Roche). 400 µm slices were resliced to 50 µm sections using a vibroslicer (Vibratome 1500, Warner instruments). Slices were stored in PBS (pH 7.4) at 4°C until use. For immunohistochemical

analysis, slices were permeabilized with 0.5 % Triton X-100 in PBS (pH 7.4; 10 min). Slices were then blocked in 4% normal goat serum in PBS (pH 7.4) for 1-hour at RT. Slices were incubated with primary antibodies of interest (overnight, 4 °C), washed (3 x 10 min; PBS, pH 7.4) and incubated in appropriate secondary antibodies (1 hr, RT), followed by 3 x 10 min washes (PBS, pH 7.4). All steps were performed with gentle agitation. Slices were mounted on coverslips (Histobond) in AquaPolymount mounting media (Polysciences) for imaging.

2.5.3. *Field Potential Electrophysiology*

Hippocampal slices were obtained from young adult male Sprague-Dawley rats (4-6 weeks) as previously described in section 2.5.1. *Acute Hippocampal Slice Preparation*. Briefly, rats were deeply anaesthetised with ketamine (100 mg/kg, i.p.) and decapitated by guillotine. The brains were removed the hippocampus was separated from surrounding cortical tissue. Area CA3 was removed and the hippocampi were dissected at a thickness of 400 µm, using a vibroslicer (Leica, VT1000; Figure 2-9). Slices were transferred to a porous, transparent membrane in an incubation chamber, and maintained at the interface between air and standard aCSF. Slices remained here for 30 minutes before transfer to RT for an additional 90 minutes.

Following recovery, slices were transferred to the recording chamber containing recirculating aCSF (95% O_2, 5% CO_2; 32.5 °C), superfused continuously at a rate of 2 mL/min. Baseline field excitatory postsynaptic potentials (fEPSPs) were elicited in area CA1 by stimulation of the Schaffer collateral-commissural pathway at 0.017 Hz (diphasic pulses, 0.1 ms half-wave duration) using a teflon-coated 50 µm tungsten wire monopolar electrode (A-M Systems Inc, Carlsborg, WA). Evoked responses were recorded with a glass microelectrode filled with aCSF (1.9-2.9 MΩ) and placed in stratum radiatum of area CA1 (approximately 300 µm from stimulating electrode; Figure 2-10). During periods of baseline recording the stimulation intensity was adjusted to elicit a fEPSP with an initial slope value of 40% of the maximum elicited when delivering 200 µA of current. Drugs, including sAPPα (1 nM) and IEM-1460 (100 µM), were bath-applied by switching to an identical preheated and oxygenated aCSF solution that contained the compound of interest. IEM-1460 was delivered 20 minutes prior to, and during, sAPPα administration and continued 10 min post-TBS. Non-saturated LTP was induced by applying a half-maximal train of standard theta burst stimulation (TBS; 5 trains of 5 pulses at 100 Hz delivered at 200 ms intervals) at baseline stimulus intensity (Raymond et al., 2000; Mockett et al., 2019).

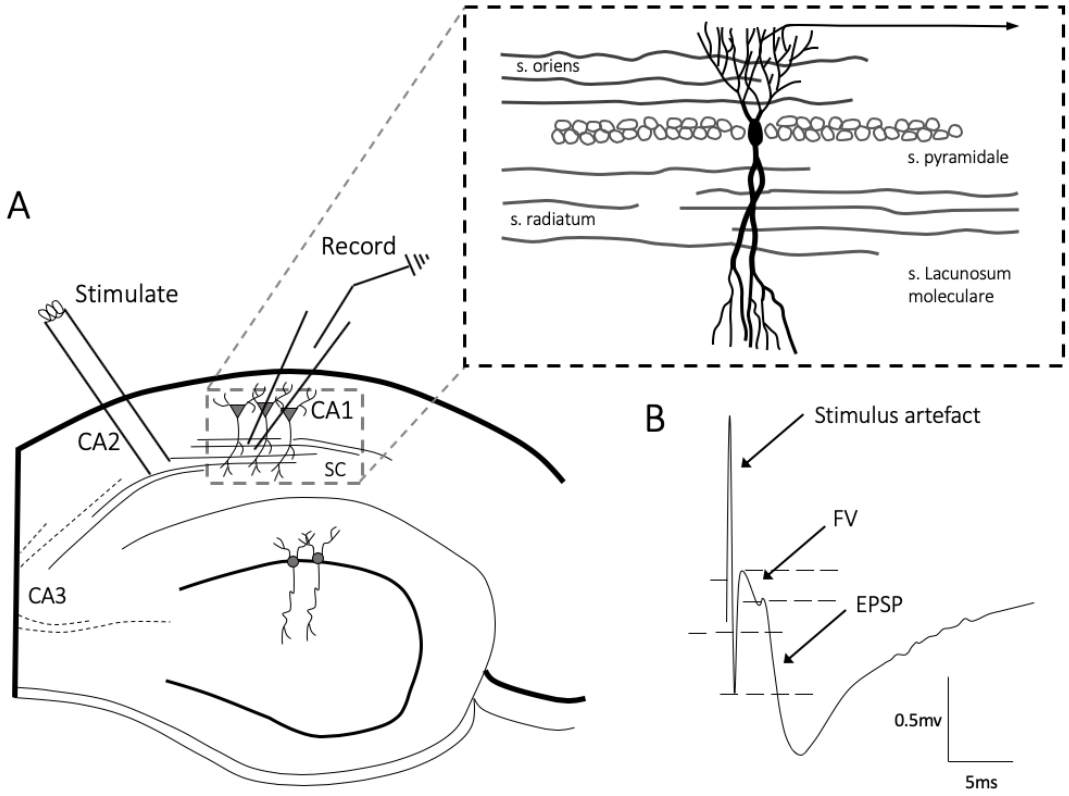


Figure 2-10 | Hippocampal slice diagram. A) Illustration of a transverse hippocampal section used in electrophysiological experiments. Inset image shows magnification of area stimulation and recording area CA1. s. = stratum. B) Representative extracellular waveform. Electrical stimulation of CA1 Schaffer collaterals elicits a stimulus artefact, followed by a presynaptic population spike or fibre volley (FV). The slope of the negative-going field excitatory postsynaptic potential (EPSP) corresponds to depolarising synaptic currents.

2.6. Microscopy and data analysis

2.6.1. *Sample Choice and Blinding*

The primary criterion for the selection of cells for imaging was the presence of a healthy appearance and proximity to neighbouring cells. Health was determined by MAP2 immunofluorescence with an absence of dendritic fragmentation or swelling. Additionally, DAPI-stained nuclei were examined for signs of fragmentation or condensation (refer Appendix 2. *Cell health*). Cells were selected which were not completely isolated from neighbouring cells but presented clearly traceable, unobstructed dendrites. Oftentimes cells closest to the outer edge of the dish were selected due to these criteria. A minimum of ten cells per dish was selected in this manner for imaging.

2.6.2. *Light Microscopy*

For experiments examining the expression of a protein using immunofluorescence, images were acquired using an Olympus IX71 inverted light microscope using a 40x/0.6-N.A objective (LUCPLFLN). The images were captured using a Hamamatsu Orca-AG camera (C4742-80-12AG) in 1024 x 1024 pixel 8-bit mode and saved as *.tif* files.

2.6.3. *Image Analysis*

To quantify immunofluorescence, neurons were outlined using ImageJ (refer Appendix 1.1. *Quantifying Arc protein expression in primary hippocampal neurons*). An 'integrated intensity/neuronal area' value was generated for each cell and somatic compartment, including all dendrites up until intersection with neighbouring dendrites. This value was corrected for average background fluorescence by subtracting average background fluorescence. Dendritic fluorescence was determined by subtracting corrected somatic values from whole cell values. For the analysis of primary and secondary dendrites, gray values were sampled at 0.5 µm increments, and the averages were binned into 50- and 25 µm segments, respectively. Statistical analysis was achieved by averaging each dendritic segment per cell, for a total cell average. For experiments examining pCREB expression in acute hippocampal slices (refer Appendix 1.2. *Quantifying pCREB protein in primary hippocampal slices*), DAPI was used to define a 'mask' around the nuclear layer and pCREB was measured as 'integrated intensity/DAPI area.' For experiments examining Arc expression in acute hippocampal slices (refer Appendix 1.2.1. *Quantifying Arc Protein Signal in Hippocampal Slices*), area CA1 was defined by a square area adjacent to the hippocampal fissure (encompassing both the pyramidal cell layer and stratum radiatum) and an integrated intensity/area' value was generated for each slice.

To quantify the PLA signal within the cultured neurons, a custom made ImageJ script created by Maximillian Heumüller (Max Planck Institute for Brain Research, Frankfurt) was used (tom Dieck et al., 2015; refer Appendix 1.3. *Quantifying PLA signal in primary hippocampal neurons*). In brief, images were separated into single channel images and the PLA signal threshold was manually defined by averaging the optimal value for several images. This value was applied to the PLA channel, and the area of the punctate signal was measured. The PLA signal was expressed relative to neuronal size as defined by the MAP2 signal which was manually outlined and isolated. The MAP2 mask was dilated by 2 pixels to encompass signal present in spines. For experiments examining the somatic signal, the analysis was conducted as above except that the somatic compartment was manually isolated from dendrites and surrounding cells. The somatic analysis for BioPLAy and PLA for the detection of subunit

dimers included a step to remove PLA detected within the nucleus, as these experiments showed non-specific PLA signal. In order to analyse the dendritic PLA signal, all traceable dendrites to the main neuron were measured 50 µm proximal from the soma, and straightened with the 'straighten' plug-in in ImageJ, and the PLA signal was analysed as above.

To analyse the proximity of PLA signal to the synaptic marker synapsin-1, the ImageJ plugin Just Another Colocalization Plugin (JACoP) was used (refer Appendix 1.4. *Quantifying Colocalization of PLA Signal Within Synapses*). In brief, images were separated into single channel images and the proximal 50 µm of MAP2 and PLA signal was isolated. Average PLA and synapsin-1 signal threshold values were determined for all treatment groups and applied within the JACoP plugin. In order to determine colocalization, the Mander's overlap coefficient was used to generate a value proportionate to the level of overlap of PLA on synapsin-1 signal. Here, a value of -1 indicates negative correlation, 0 indicates no correlation and +1 indicates a positive correlation. In addition, the proximity of PLA puncta to synapsin-1 signal was determined by manual measurements of the centre of each PLA puncta (determined by the brightest pixel) to the centre of mass of the nearest synapsin-1 puncta. PLA puncta within 0-2, 2-4, and > 4 µm from the synapsin-1 centre of mass were considered synaptic, extrasynaptic, and non-synaptic, respectively.

2.6.4. *Statistical Analysis*

For RT-qPCR data, data exhibited a normal distribution (Shapiro–Wilk normality test), significance was assessed using Student's t-tests where $p < 0.05$ was accepted as significantly different. Data were normalized to the control gene HPRT and expressed relative to no-drug control.

Statistics for all immunocytochemistry experiments were performed using Kruskal–Wallis one-way ANOVA followed by Dunn's multiple comparisons test. Normal distribution of data was determined (D'Agostino and Pearson omnibus normality test). Outliers within the raw data sets were detected using the Grubb's test. Data for all slice work exhibited a normal distribution (Shapiro–Wilk normality test). Experiments examining pCREB expression were analysed by use of Student's t-tests, and experiments analysing Arc protein expression were analysed using one-way ANOVA followed by Šidák's multiple comparisons test. Data are expressed as fold change relative to control values.

For electrophysiology experiments, the baseline response value was calculated by averaging the responses over the final 10 min before LTP induction. All responses throughout the recording were normalised to the baseline value before application of IEM-1460 and expressed as a percentage of baseline. Normal distribution of data was determined by D'Agostino and Pearson omnibus normality test. Statistical differences between experimental groups was performed on data

normalised to the average of baseline 10 minutes before TBS, and determined by one-way ANOVA with Tukey's multiple comparisons tests, where appropriate.

To observe the effect of treatment on the expression of proteins at the cell surface, data were normalised to the experimental control condition. Outliers within the raw data sets were detected using the Grubb's test, and identified candidates were removed if significantly different from the data set, following visual analysis of the PLA channel. For all data sets, D'Agostino and Pearson omnibus normality tests were used to determine whether the data was normally distributed. Data from ≥ 3 experiments was amalgamated and significance was by assessed one-sample t-tests (relative to a theoretical mean of 1) or Mann-Whitney two-tailed u-tests (Girard et al., 2007; Boos and Stefanski, 2013; tom Dieck, et al., 2015). For data examining the proportion of proteins within synaptic domains, normality was determined by Shapiro-Wilk normality test. Significance was assessed by two-way ANOVA and Šidák's multiple comparisons effect.

Chapter 3: Results

3.1. *Characterization of Primary Hippocampal Cultures*

Previous research investigating the mechanisms of learning and memory have utilized primary hippocampal and cortical cultures as a means of isolating neurons for analysis of biological properties related to cellular structure and function. A common procedure for producing these cultures involves the manual dissection and isolation of cortical or subcortical structures from rodent or murine pups, dissociation of the cellular profile, and incubation and maintenance of plated cells for up to four weeks or longer (Banker and Goslin, 1998). Comparisons of *in vitro* cell cultures to the likes of *ex vivo* tissue sections have been continually present throughout the literature as ongoing research is performed with both preparations. While brain sections have been regarded as an exceptional preparation for maintaining local synaptic circuitry and preserving brain architecture (Cho et al., 2007), primary cultures have shown an increasing advantage as a convenient and viable technique for simulating the molecular microenvironment of complex *in vivo* tissue, and allowing greater spatial resolution (Millet and Gillette, 2012). This thesis aimed to examine the regulation and expression of IEGs and glutamate receptors in primary hippocampal neurons in culture, in response to the neurotrophic protein, sAPPα. Therefore, before these experiments begin, we first aimed to describe the cellular and molecular environment present in these cultures.

3.1.1. *Growth and Development*

Primary hippocampal cultures were prepared from PD 0-1 rat pups and maintained in culture for 21–27 DIV prior to use (refer section 2.1. *Primary Neuronal Culture Preparation*). While synaptic development has been shown to occur within a week *in vitro*, full maturation and complexity of both pre- and post-synaptic complements does not occur until > 21 days *in vitro* (Dotti et al., 1988; Fletcher et al., 1991; Fletcher et al., 1994; Grabrucker et al., 2009). Development of cultures was documented as the cells matured using phase contrast microscopy. In accordance with previous studies (Banker and Cowan, 1977; Dotti et al., 1988; van Spronsen et al., 2013), immediately following plating, cells were observed as small spherical bodies beginning to adhere to the Poly-D-lysine coated glass coverslip (Figure 3-1A). By DIV 2, cell bodies began to produce minor processes with many extending primary projections. Over the following 7 days, dendritic arborization increased in size and complexity (Figure 3-1B), and throughout the next two weeks cells continued to project dendrites and axons (Figure 3-1C), increasing the complexity of the network and covering a majority of the culture surface (Figure 3-1D).

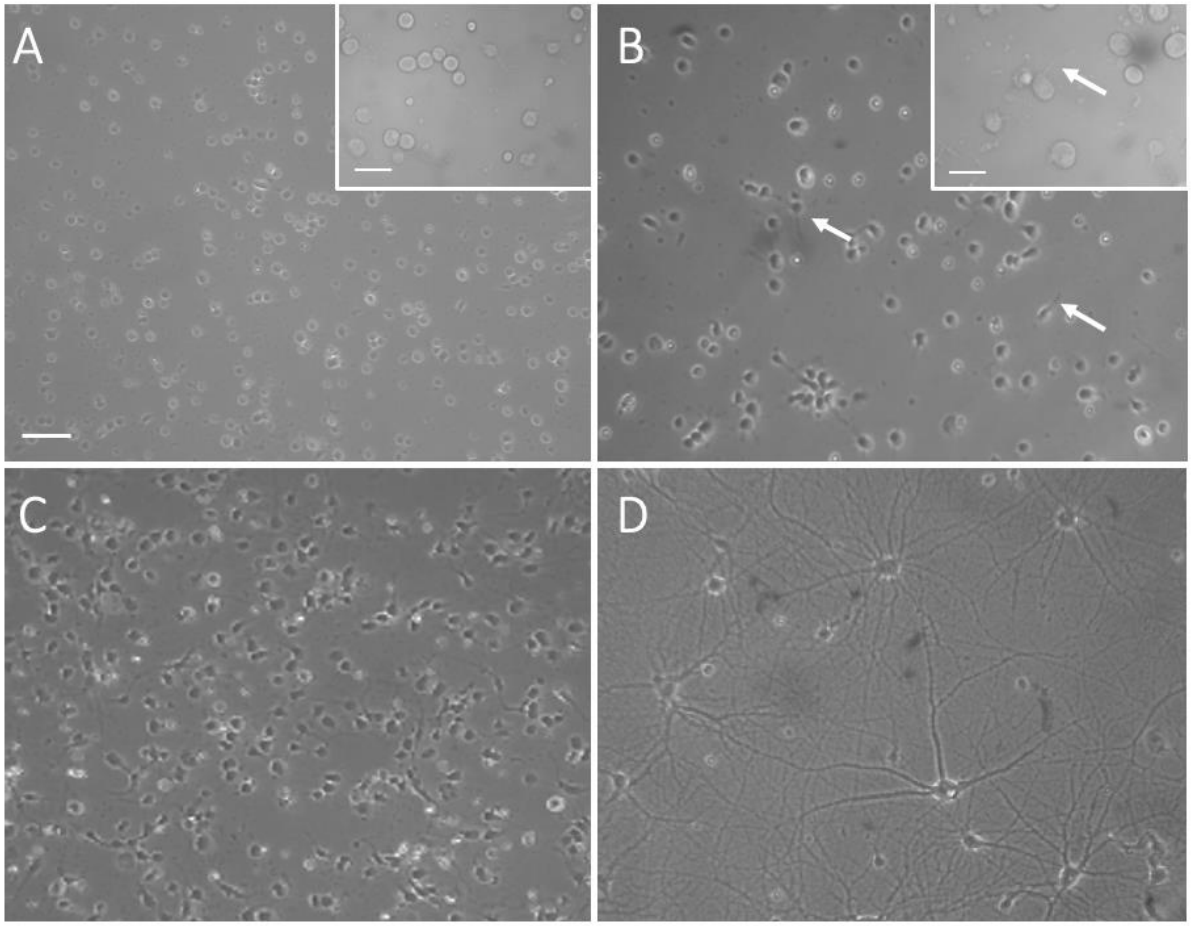


Figure 3-1 | Temporal development of hippocampal cells in primary culture. Phase contrast images of primary hippocampal cells in culture (at a density of 4 x 10^4 cells/cm^2) imaged at various time points following initial plating. Seeded cell bodies (A, upper right inset) begin to send out primary projections and minor neurites by DIV 2 (B, indicated by white arrows), and by DIV 7 have produced multiple projections (C). By DIV 24, a dense and complex arborization of dendrites is seen extending from a large soma (D). Scale bar = 100 µm (10 x), scale bar (inset images) = 50 µm (A), 20 µm (B; 40 x).

3.1.2. *Immunofluorescent Detection of Primary Neuron Development*

In addition to examining cell development by phase contrast microscopy, it is also possible to observe development by probing cells for key proteins using immunocytochemistry. This method allows for finer observation of neuronal growth by examining key proteins in cells of interest. By probing for the neuron specific protein microtubule-associated protein 2 (MAP2; Caceres et al., 1984), we are able to distinguish MAP2-postive immunofluorescence concentrated in the soma of neurons at the instant of cell plating (0 hours; Figure 3-2A; Bernhardt and Matus, 1984). At this stage, it is expected that cells develop a lamellipodia around the circumference of the cell body (Dotti et al., 1988), containing predominantly actin filaments. By 24-48 hours we can begin to distinguish MAP2- and tau-positive minor neurites (Figure 3-2B-C). These neurites appear cylindrical, yet have expanded, flattened, growth cones at their tips. Once established these minor neurites appear rather stable, with many continuing to develop (Dotti et al., 1988).

As is common for hippocampal neurons in culture, by 72 hours the primary axon arose as a branch from a short process which had dendritic characteristics, including MAP2-immunoreactivity. This tau-positive axon differentiates and can be marked as the establishment of neuronal polarity (Yogev and Shen, 2017; Figure 3-2D). This polarity can be observed through a marked increase in its growth rate, a decrease in MAP2-immunoreactivity, and an increase in tau-immunoreactivity. From this stage on, the primary axon grows rapidly, while minor processes begin to elongate and take on the appearance of dendrites. This development continues throughout DIV 3-8 (Figure 3-2E-G), while dendrites continue to grow and develop, however at a slower rate than axons (LeBrasseur, 2005; Polleux and Snider, 2010).

By DIV 14 additional axons can be observed as branching collaterals emerging from the primary axonal process. Around this time, increased observations of *en passant* boutons are possible, as axons began to make close associations with neighbouring dendrites. Many of these associations appear as bulb-like protrusions from the axonal process, likely indicating the presence of presynaptic boutons, and either the formation of immature synapses or the presence of mature synapses (Reilly et al., 2011; Schedin-Weiss et al., 2016; Chéreau et al., 2017; Figure 3-2G). By DIV 21-24, neurons reach full dendritic and axonal arborization and growth begins to plateau (van Spronsen et al., 2013; Sahu et al., 2019; Figure 3-2H). Associations between axons and dendrites appear to become more refined, increasing in number along greater lengths of the dendrite (Figure 3-2F).

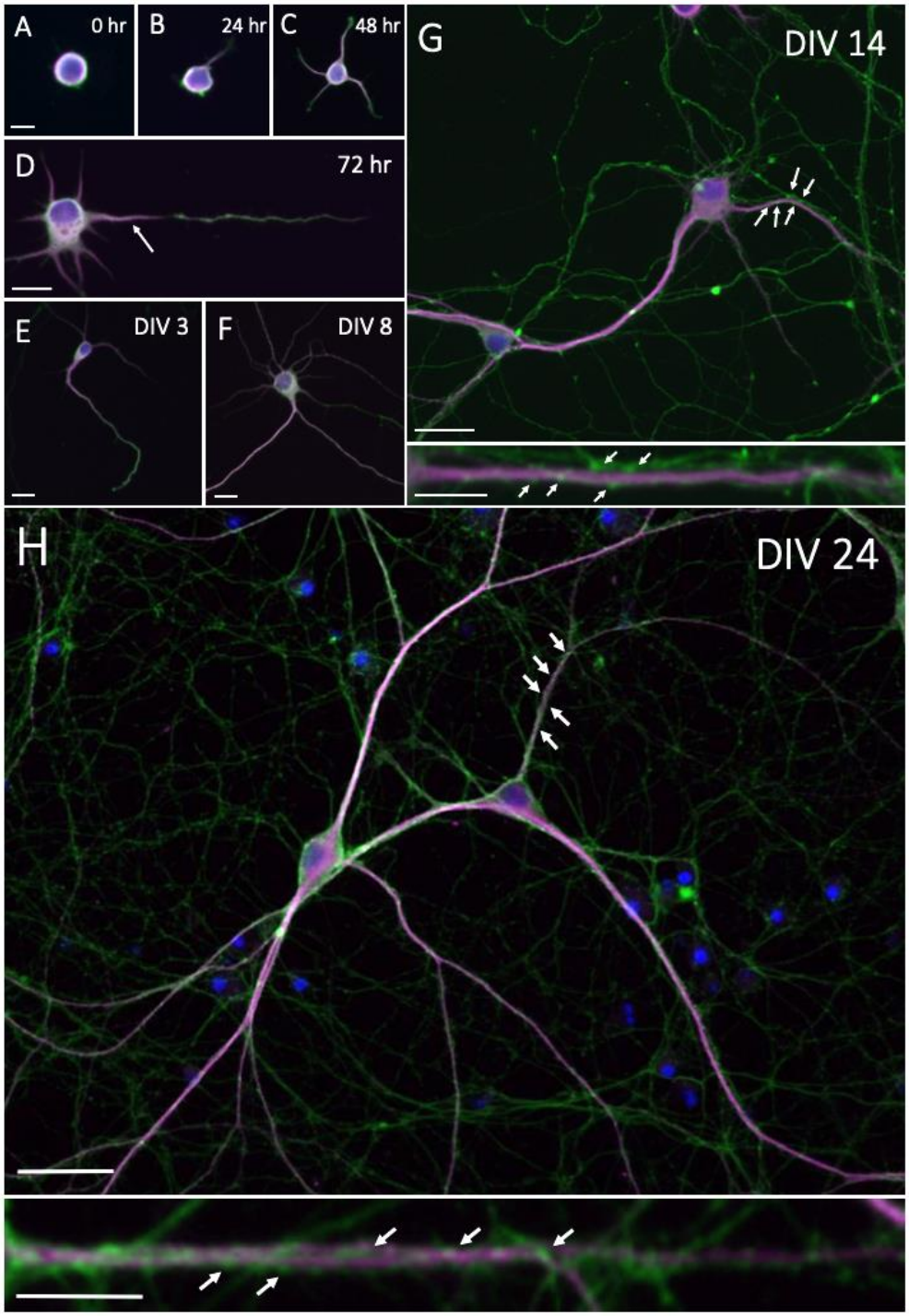


Figure 3-2 | Temporal development of MAP2- and Tau-positive neurites. Immunocytochemistry images of primary hippocampal cells in culture (40 x 100 cells/cm^2) imaged at various time points following initial plating. Seeded cell bodies (A) begin to send out a MAP2-postive neurite within 24 hours (B). Additional neurites form within 48 hours, becoming tau-positive (C). A primary neurite becomes primarily tau-positive by 72 hours developing into an axon (D) and MAP2-positive axon-initial segment (arrow). By DIV 3 MAP2-positive and tau-positive neurites extend to become dendrites and axon, respectively (E). By DIV14 the formation of *en passant* boutons is observable (arrows, G). Inset image shows magnified section of dendritic and axonal proximity. Dendritic and axonal growth continues through DIV 24, with the persistence of *en passant* boutons (arrows, H). Inset image shows magnified section of dendritic and axonal proximity. Arrows indicate close associations of tau-positive axons and MAP2-positive dendrites. Images show neurons (MAP2, ■), nucleus (DAPI, ■), and axons (Tau, ■). n = 1. Scale bars = 20 µm (A-F), 50 µm (G-H), inset image = 10 µm.

3.1.3. *Cellular Populations Within Primary Hippocampal Cell Cultures*

In order to further understand the cell types present within our primary hippocampal co-cultures, we probed our cultures with immunocytochemical markers of cell type-specific proteins. While our experimental investigations will focus primarily on the expression of proteins in neurons, it is important to note that in healthy co-cultures such as those produced using our protocol, a mixture of both neuronal and glial cells will be present. Due to the proliferative capability of glial cells, left long enough, co-cultures will become overgrown with glial cells, an occurrence which may obscure or otherwise affect downstream analysis of molecular changes (Lauer et al., 2002), thus it was important to establish the expression of these cell types before downstream analysis. Many methods have been developed to circumvent this, including addition of the anti-mitotic drugs such as cytarabine (Ara-C), a pyrimidine antimetabolite additive that acts to inhibit DNA synthesis and thus the division of proliferative cells such as glia (Delivopoulos and Murray, 2011). However, methods such as these have been shown to have adverse effects on neurons (Wallace and Johnson, 1989; Martin et al., 1990). It is well established that the presence of glia *in vitro* and *in vivo* is necessary for the development and function of neurons (Helmuth, 2001; Ullian et al., 2001). Specifically, several astrocyte-derived signals have been described as essential for the induction of neuronal neuritogenesis and synaptogenesis (Mauch et al., 2001; Medina and Tabernero, 2002; Haydon and Carmignoto, 2006; Barker et al., 2008; Bertrand et al., 2011; Yates, 2012). Our protocol for the culturing of primary hippocampal neurons involves the maintenance of cultures in serum-free Neurobasal-A media and B27 supplement which support the growth and development of neurons but does not directly support glial proliferation (Brewer et al., 1993). Therefore, in order to provide an initial support of glial cell growth, within the first four days, co-cultures were supplemented with conditioned media derived from glial-only cultures (refer Section 2.1.3. *Production of Conditioned Media for Cell Culture Maintenance*). Cultures were also supplemented with a small percentage of media from neuronal and glial co-cultures, in order to account for any secreted factors that may require the presence of both cell types. This allows for initial proliferation and support from glial cells, after which media supplementation comes solely from Neurobasal-A media, B27 and GlutaMAX only. This method has been shown to enhance survival of neurons in co-culture (Conde Guerri et al., 1989; Shi et al., 2013).

Here, we have probed our primary hippocampal cultures (DIV21-24) with immunocytochemical markers such as MAP2, or the glial fibrillary acidic protein (GFAP), present in astrocytic cells (Eng et al., 2000). These markers have been shown to be reliable indicators of neuron and astrocyte morphology, respectively, through their continued expression *in vitro* (Caceres et al., 1984; Sofroniew and Vinters, 2010). As shown by Figure 3-3, our primary hippocampal cell cultures show distinct populations of both MAP2- (Figure 3-3A,C) and GFAP-positive (Figure 3-3B-C) cells,

expressing typical neuronal and glial cell morphology (Müller et al., 2015). While the ratio of astrocytes per neuron has been shown to be 1:1.5 in the rodent hippocampus (Keller et al., 2018), this method provided a 1:1 ratio of neurons to astrocytes by DIV24 (cell counts of MAP2- and GFAP-positive cells; *n* = 1 experiment), similar to previous observations in culture (Dawes et al., 2018; Gunhanlar et al., 2018). In line with observations described in the literature, these co-cultures appeared to form close associations with GluA1-positive neurons (Figure 3-3D), which may indicate the formation of *in vitro* tripartite synapses (Todd et al., 2013; Robertson et al., 2014).

In addition to this, we have further identified subpopulations of granule cells within our neuronal population (Figure 3-3E). This population was isolated visually by use of antibodies against the granule cell specific transcription factor Prospero Homeobox 1 (Prox1; Lavado et al., 2010; Iwano et al., 2012), and indicates that our cultures contain distinct populations of Prox1-positive and -negative neurons. Identification of these populations provides information which may supplement downstream analysis and interpretation of results. Prox1-positive neurons accounted for 30% of MAP2-positive neurons in our cultures (cell counts of PROX1-negative and PROX1-positive cells; *n* = 1 experiment), with the remaining 70% of neurons able to be attributed to excitatory and inhibitory populations, similar to past observations (Soumier et al., 2009; Digilio et al., 2015). Interestingly, observations from past literature have shown population estimates of almost 1:1 granule to pyramidal cells when obtained from young postnatal (PD 0) mouse pups (Wu et al., 2015). While curious, this may arise from developmental differences between species.

Furthermore, as shown in Figure 3-3A, MAP2 staining shows continuous and robust signal, indicating the presence of a healthy neuron. In unhealthy neurons, MAP2 expression is fragmented in the dendrites and can take on a 'beads on a string' appearance due to cellular ‘blebbing’ of the membrane, a reliable indicator of cell stress (Charras, 2008; Appendix Figure A-15). Alternatively, unhealthy astrocytes have been shown to be double-stained by both GFAP and MAP2, and represents a permanent change in reactive astrocytes (Geisert et al., 1990; Schinstine and Iacovitti, 1996). In addition to this, the use of the nuclear stain 4′,6-diamidino-2-phenylindole (DAPI) can be used to identify cell bodies, as well as the presence of a fragmented, condensed, or swollen nuclei, also an indication of poor health or cell stress (Dini et al., 1996). While few unhealthy cells are to be expected in long-term maintenance of primary cultures, cultures consistently had a very low level of unhealthy cells as determined by the above criteria.

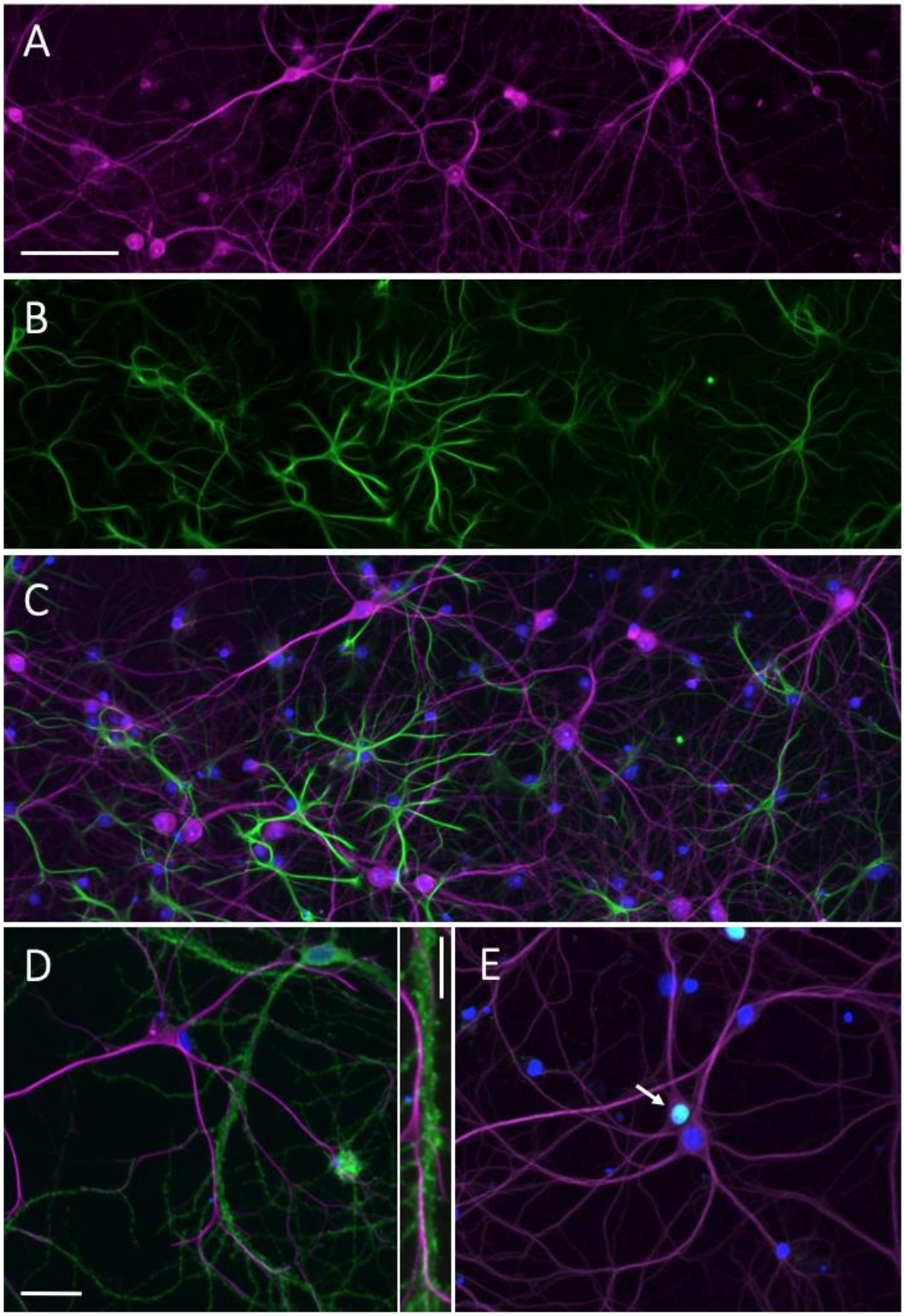


Figure 3-3 | Primary Hippocampal cultures contain Glial and Neuronal populations. Representative images show A) MAP2-positive neurons (■), B) GFAP-positive astrocytes (■), C) the close association between neurons and astrocytes throughout the cultures, and D) close associations with GluA1-positive dendrites (GFAP, ■; GluA1, ■). D) Populations of Prox1-postive (arrow) granule cells are present in our hippocampal cultures (MAP2, ■; Prox1, ■, DAPI, ■). *n* = 1 experiment. Scale bars = 100 µm (A-C), 50 µm (D-E), inset images = 10 µm.

3.1.4. *Identification of Pre- and Post-Synaptic Markers of Mature Synapses in Neuronal Cultures*

Synaptic transmission is a fundamental property of neurons, including those grown in culture (Vogt et al., 1995; Maximov et al., 2007). Communication between cells occurs primarily at synapses, which consist of pre- and postsynaptic domains separated by a 10–20 nm wide extracellular space, called the synaptic cleft. The most direct approach for the visualisation of synapses is the use of immunocytochemical markers for both pre- and postsynaptic proteins (Biederer and Scheiffele, 2007). Presynaptic proteins such as synaptophysin-1, a vesicle-associated protein present along axons (Fletcher et al., 1991; Di Liegro et al., 1995; Kwon and Chapman, 2011), and the postsynaptic excitatory glutamate receptor subunits GluA1 and GluA2, are reliable markers of mature and active postsynaptic domains (Pickard et al., 2000; Jacob and Weinberg, 2015). Single- and dual-labelling of these proteins in culture allows for visualisation of synapses via conventional fluorescence microscopy (Pickard et al., 2000; Verstraelen et al., 2018). At this resolution, dual labelling of pre- and postsynaptic proteins results in the coalescence of fluorescent signals, at points at which both proteins are present in close proximity (approximately 200-500 nm; Huang et al., 2009; Dzyubenko et al., 2016). Therefore, while single-labelling immunofluorescence can give an indication of protein content within a cell, dual-labelling of synaptophysin-1 and GluA1 or GluA2 gives an indication of pre- and postsynaptic terminals in close contact.

By one-week *in vitro* cells will begin to produce endogenous action potentials and synapses will begin to form, alongside the presence of immature dendritic spines (Dichter et al., 1983; Basarsky et al., 1994; Papa et al., 1995). From DIV8, hippocampal neurons grown in culture express the GluA1 protein (Zhang et al., 2017) and contribute to synaptic currents (Shi et al., 2010). From DIV8 onwards, spines continue to mature and the number of both excitatory and inhibitory synapses continues to increase (Grabrucker et al., 2009), as the length and number of dendrites also increases (Harrill et al., 2015). By DIV 21 a majority of synapses contain dual immunoreactivity for pre- and postsynaptic proteins (Grabrucker et al., 2009), reaching a relatively stable plateau.

Here, we probed our cultures (DIV 8) for GluA1, and found a small but notable presence of GluA1 protein within both the soma and dendrites (Figure 3-4A). We further examined GluA1 (Figure 3-4B) and GluA2 (Figure 3-4C) in DIV 21 hippocampal neurons which were present to a greater extent in both somatic and dendritic compartments, in line with previous observations (Richmond et al., 1996). DIV 21-27 neurons were also found to be positive for the presynaptic marker synaptophysin-1 (Figure 3-4D), which colocalized with both GluA1 (Figure 3-4E) and GluA2 (Figure 3-4F), indicating the presence active synapses in our cultures.

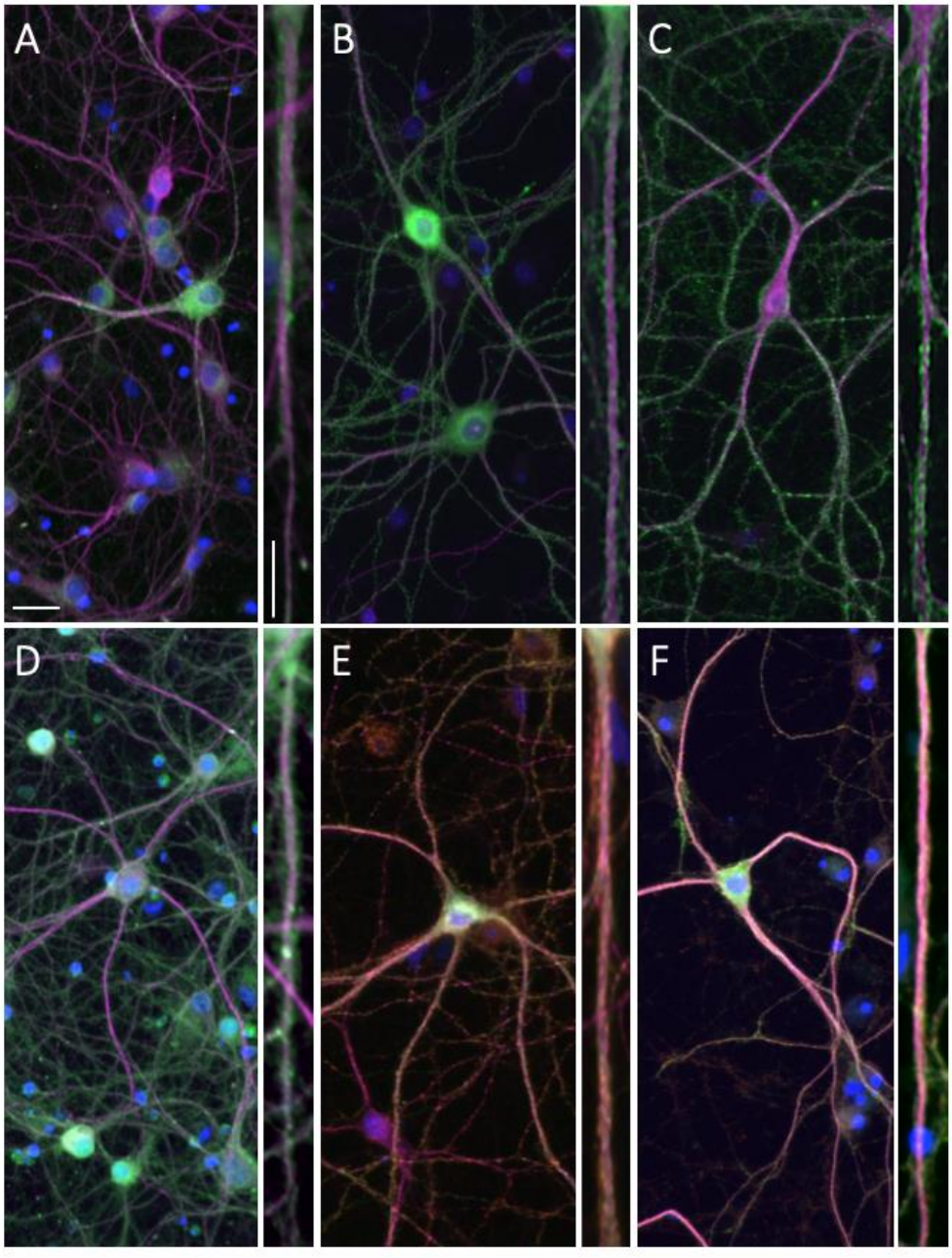


Figure 3-4 | Development of synapses in vitro. Representative images show hippocampal neurons stained for A) GluA1 (■, DIV 8), B) GluA1 (■, DIV 21-27), C) Synaptophysin-1 (■, DIV 21-27), D) GluA2 (■, DIV 21-27), E) the colocalization of both synaptophysin-1 (■) and GluA1 (■), and F) the colocalization of both synaptophysin-1 (■) and GluA2 (■). All images show neuronal architecture with MAP2 (■) and DAPI (■). *n* = 1 experiment. Scale bar = 50 µm, inset images = 20 µm

3.2. *Observations from the Culturing of Adult Cortical Mouse Neurons*

The culturing of tissue from both embryonic and early postnatal rodents has been shown to result in successful growth of both neuronal and glial populations. While the use of young tissue provides an easy and effective means to isolate primary neurons, these developmental stages may not accurately represent later stages of development or disease progression. However, successful culturing of neurons from adult animals has been historically difficult, and at times described as impossible, yet few groups have found success in isolating neurons and glia (Brewer, 1997; Eide and McMurray, 2005; Brewer and Torricelli, 2007; Sun et al., 2017), dorsal root ganglia (Mollá et al., 2017), and neural progenitor cells (Palmer et al., 1999; Ray and Gage, 2006; Babu et al., 2007) from adult tissue.

Here we aimed to establish a protocol for the preparation of adult neuronal cultures. Primary cortical cultures were prepared from 9-month-old wild-type (WT) and APPswe/PS1ΔE9 C57/Bl6 (APP/PS1) mice and maintained in culture for 9-14 days DIV prior to use. Adaptions were made to our current protocol for culturing postnatal neurons (refer section 2.2 *Adult Primary Neuronal Culture Preparation*; Eide and McMurray, 2005; Brewer and Torricelli, 2007).

3.2.1. *Growth and Development of Adult Neuronal Cultures*

Similar to cultures derived from early postnatal tissue, development of adult neuronal cultures was documented as the cells matured using phase contrast microscopy. In line with observations from Eide and McMurray, (2005) and Brewer and Torricelli, (2007), immediately following plating, cells were observed as small spherical bodies and pyramidal soma, beginning to adhere to the poly-D coated glass coverslip (Figure 3-5A). After roughly 5-7 days adult cellular 'colonies' appeared (Figure 3-5B), and continued to extend processes (Figure 3-5C). Some neuron-like cells were observed clustering together with glia (Figure 3-5D), however without confirmation by use of immunocytochemical markers, these observations are purely morphological. While some groups have reported maintenance of adult cortical neurons for as long as 3 weeks (Ross et al., 2018), by DIV 14, neurons began to deteriorate, appearing as clusters of bright spheres, indicating detachment from the glass coverslip and cell death. The youngest cultures (DIV 9) were thus fixed and prepared for immunocytochemistry.

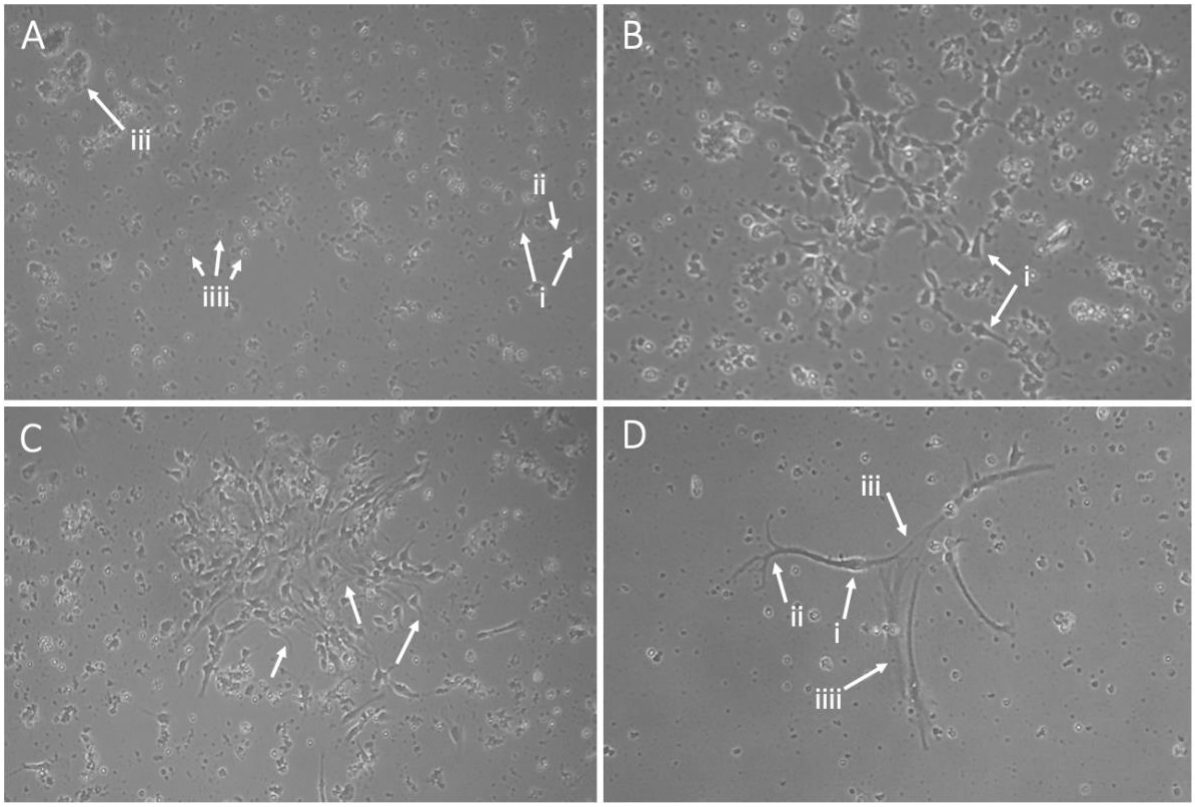


Figure 3-5| Adult mouse culture growth and development. A) By DIV 3, cultures begin to show the presence of neurons (i) with immature neurites (ii). DIV 3 cultures also show some cellular debris (iii), and many unadhered dead cells (iiii). B) by DIV 5 'colonies' of cells can be observed with neurons and small neurites (i). C) these colonies continue to grow and neurites continue to extend and develop into identifiable dendrite-like branches (white arrows). Neuron development and the presence of glia is more obvious in D) smaller colonies where neurons (i) show multi-branching dendrites (ii) and axons (iii), in close proximity to fusiform shaped astrocytes (iiii). *n* = 1 experiment. Scale bar = 50 µm.

3.2.2. *Cellular Populations in Adult Primary Cortical Cultures*

In line with our previous work, utilizing neuronal tissue obtained from PD 0-1 rat pups, we aimed to examine the populations of neurons and astrocytes in our adult mouse cultures. Immunocytochemistry was used targeting the neuronal marker MAP2 and the astrocyte marker GFAP. Here the addition of DAPI was used as an indicator of nuclear bodies present in culture which may not also express positive for MAP2 or GFAP. Through this, we observed conservation of the 'colonies' seen via phase contrast microscopy. Interestingly, in dishes double labelled for GFAP and MAP2, majority of the colonies were positive for GFAP with few MAP2-positive neurons present. Astrocytes showed typical morphology for those cultured from adult tissue (Sun et al., 2017), with a majority adopting polygonal/fusiform or flat shaped cell bodies, although some astrocytes formed monopolar or bipolar morphology. A majority of neurons present showed a typically small soma, with 1-2 MAP2-positive primary projections (Figure 3-6).

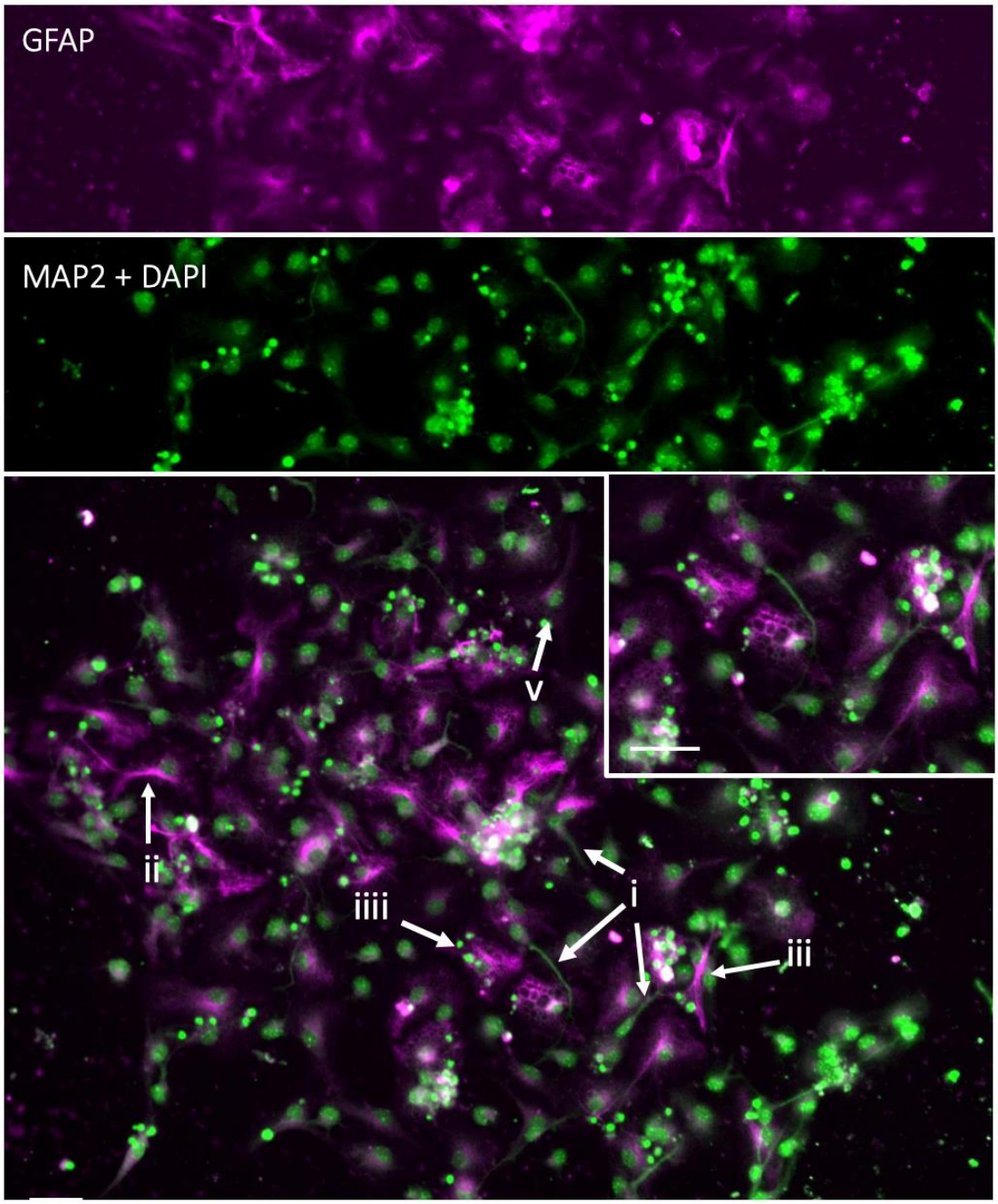


Figure 3-6| Cellular populations in adult cortical cultures. Immunocytochemistry showed populations of i) MAP2- and DAPI-positive neurons (■), and GFAP-positive (■) ii) monopolar, iii) bipolar, iiii) polygonal, and v) flat astrocytes (DIV 9). Inset image shows digital magnification of neurons and astrocytes. *n* = 1 experiment. Scale bar = 50 µm.

3.2.3. *Cultured Wild-Type and APP/PS1 Adult Mouse Neurons Express GluA1*

Secondary to examining the presence of cell populations within our adult mouse cultures, we further aimed to examine whether the cells present in our cultures express appropriate glutamate receptors. GluA1 is present in the mouse cerebral cortex and associates strongly with immunocytochemical markers for neurons (Carino et al., 2012; Maheshwari et al., 2012), glial cells (Bender et al., 2020), and reactive astrocytes (Hefferan et al., 2007). Importantly, past observations have shown high immunoreactivity for pre- and postsynaptic markers synapsin-1 and PSD-95, respectively, by 3 weeks *in vitro* (Ross et al., 2018), indicating adult-derived neurons are capable of maintaining the regeneration of synapses in culture.

As mentioned in section 3.1.4. *Identification of Pre- and Post-Synaptic Markers of Mature Synapses in Neuronal Cultures*, neurons obtained from embryonic or young postnatal animals have previously been shown to express synaptic markers, including GluA1, from 8 days onwards (Richmond et al., 1996). Here, we have obtained cells from much older tissue, with perhaps slower regenerative abilities than younger tissue, and maintained for only 9 days *in vitro*. In face of this, when examining GluA1 expression under permeabilized conditions, our cultures showed many GluA1- and MAP2-positive cells in cultures obtained from both WT and APP/PS1 transgenic tissue (Figure 3-7). Importantly, while many MAP2-positive cells appeared morphologically neuronal, a majority MAP2 staining expressed as showing astrocyte morphology, indicating the likely presence of GluA1-positive reactive astrocytes (Geisert et al., 1990; Schinstine and Iacovitti, 1996)

Here we aimed to establish a protocol for the preparation of adult neuronal cultures. In line with past literature, we have further described the difficulties in isolating cells for co-culture of neurons and glia. While we were successful in the isolation of MAP2- and GFAP-positive cells, the identity of these cells would require greater validation than the techniques used here. Additionally, as previously observed (Brewer, 1997), the use of Neurobasal A medium for preparation of adult neurons can sometimes result in the release of neuronal DNA following osmotic shock, possibly promoting the death of neurons in these preparations. Future attempts should aim to optimize the osmolarity of medium used to isolate adult neurons in order to promote the longevity of neurons in these cultures. For these reasons, these cultures were not ultimately used for the experimental drug treatments described throughout this thesis. However, the observations provided by this work highlights considerations when attempting this approach and provide an early proof-of-concept for future directions.

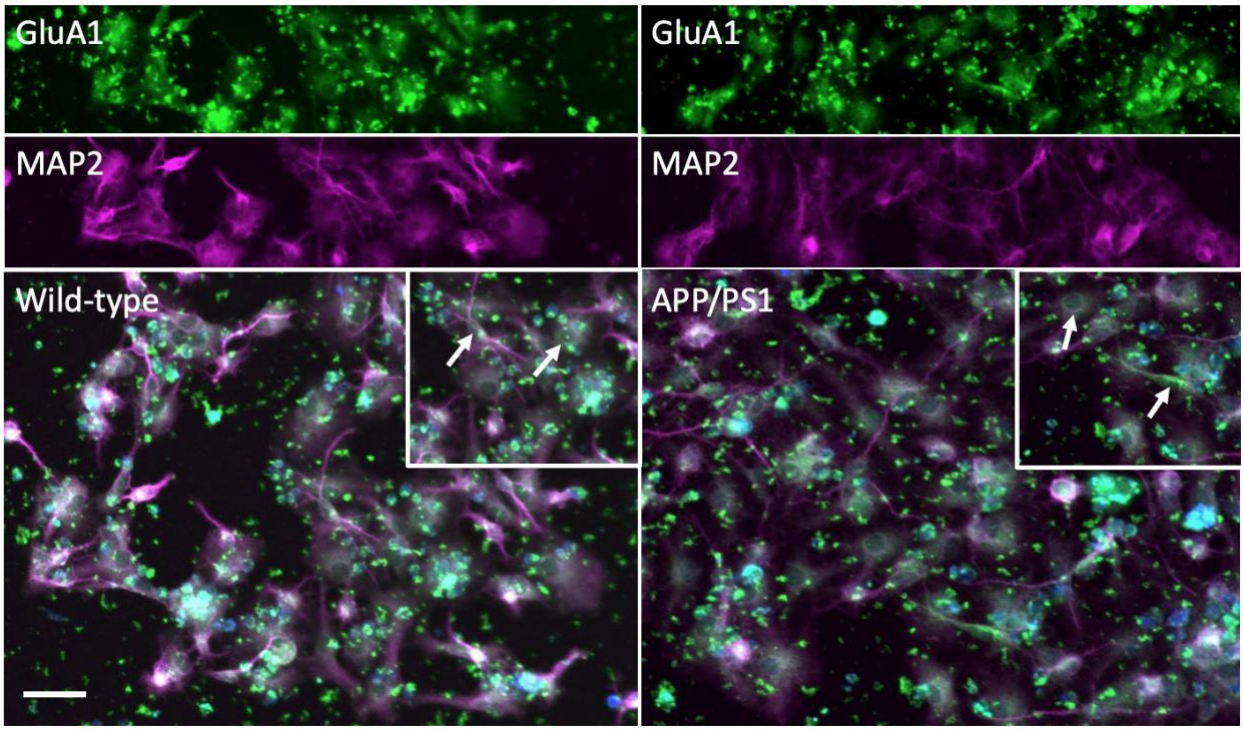


Figure 3-7| GluA1-positive cells. Immunocytochemistry showed populations of double labelled cells (DIV 9) positive for both GluA1 (■) and MAP2 (■) in tissue obtained from A) wild-type, and B) APP/PS1 transgenic mice. Upper panels show GluA1, middle panels show MAP2, and lower panels show combined GluA1 and MAP2 channels. Inset images show magnification of combined channels, arrows indicate clear GluA1-positive neurons. *n* = 1 experiment. Scale bar = 50 µm.

Chapter 4

4.1. *Examining Arc Expression in Response to sAPPα*

Historically, IEGs, and specifically Arc, has been a proven method for effectively measuring plasticity or the activation of synapses in both *in vitro* (Das et al., 2018) and *in vivo* assays of plasticity (Grinevich et al., 2009; Steward et al., 2018). Additionally, Arc has shown direct links to the expression of AMPA receptors in governing the regulation of plasticity (Rial Verde et al., 2006; Shepherd et al., 2006; Shepherd and Bear, 2011). It is recognized that both endogenous (Taylor et al., 2008) and exogenous (Richter et al., 2018) sAPPα promotes the regulation of plasticity and the expression of AMPAR (Mockett et al., 2019; Martinsson et al., 2019). Therefore, due to the inherent activity-regulated properties of Arc, initial experiments sought to utilize this, allowing for assessment of upstream and downstream processes governing sAPPα's control of plasticity and AMPAR expression in culture.

All data presented here, including additional experiments and observations is available by publication in Frontiers of Molecular Science, as part of the research topic Advances in Biomedical and Molecular Neuroscience (Livingstone et al., 2019), and can be found at https://doi.org/10.3389/fnmol.2019.00198.

4.1.1. *sAPPα Facilitates an Increase in Arc and Zif268 mRNA Expression*

Previous work has identified networks of genes associated with the treatment of hippocampal slices with sAPPα (1nM), including rapidly-induced transcription factors *JunB*, *c-Fos*, and importantly *Zif268* (Minatohara et al., 2016). Therefore, we aimed to examine whether sAPPα (1 nM) promoted an increase in *Arc* mRNA expression in primary cortical cell cultures, in a time-dependent manner, using reverse transcription quantitative polymerase chain reaction (RT-qPCR). As a positive control we also examined the expression of *Zif268,* previously shown to increase in expression following sAPPα (1 nM, 15 min) in organotypic hippocampal slices (Ryan et al., 2013), and further linked to the regulation of *Arc* mRNA *in vivo* (Penke et al., 2011). As a negative control we also examined the transcription factor Specificity Protein 2 (*SP2;* Suske, 1999). Here, we found that treatment with sAPPα facilitated a slowly developing increase in the levels of *Arc* mRNA (Figure 4-1). No significant change was found in either *Arc* or *Zif268* mRNA at 15 or 30 min of sAPPα incubation (*Arc*: 15 min: 1.27 ± 1.00, $p = 0.35$; 30 min: 1.14 ± 0.28, $p = 0.49$; *Zif268*: 15 min: 0.96 ± 0.45, $p = 0.28$; 30 min: 1.30 ± 0.68, $p = 0.30$). However, following 60- and 120-min exposure the levels of both *Arc* (60 min: 2.29 ± 1.32 $p = 0.01$; 120 min: 2.69 ± 1.53, $p \leq 0.0001$) and *Zif268* (60 min: 1.78 ± 1.02, $p = 0.01$; 120 min: 1.38 ± 0.57, $p = 0.04$; Figure 4-1)

mRNA increased significantly relative to no-drug controls. This effect was found to slowly diminish with time as both *Arc* and *Zif268* were reduced by 240 minutes (*Arc*: 1.57 ± 1.15, $p = 0.04$; *Zif268*: 1.43 ± 0.86, $p = 0.05$) and eliminated by 24 hours (*Arc*: 1.051 ± 0.14, $p = 0.45$; *Zif268*: 0.94 ± 0.043, $p = 0.32$). No significant change was found in *SP2* following any length of treatment (15 min: 1.049 ± 0.1501, $p = 0.19$; 30 min: 1.063 ± 0.15, p = 0.17; 60 min: 1.15 ± 0.24, $p = 0.10$; 120 min: 1.18 ± 0.094 $p = 0.06$; 240 min: 0.94 ± 0.043, $p = 0.33$; 24 hr: 1.032 ± 0.14, $p = 0.21$).

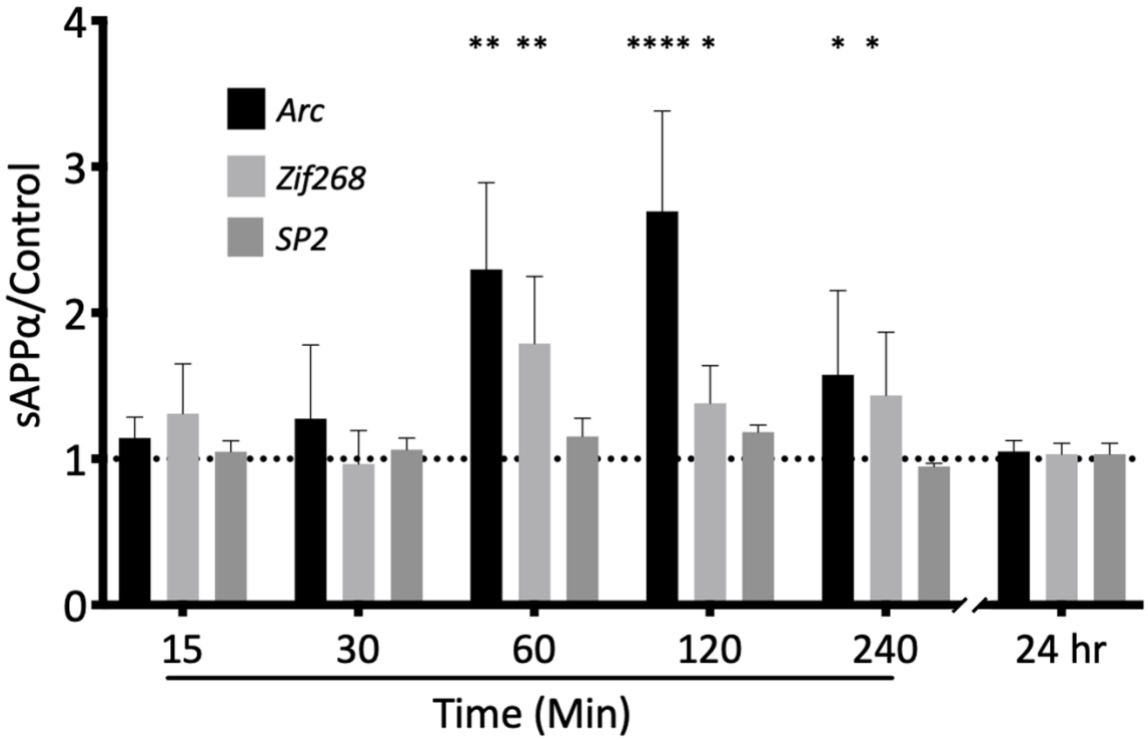


Figure 4-1 | sAPPα promotes the transcription of *Arc* and *Zif268* mRNA. RT-qPCR showed that sAPPα (1 nM) promotes an increase in the expression of *Arc* and *zif268* mRNA in primary cortical cells in culture at 60, 120 min (n = 5 wells), and 240 min (n = 4 wells) relative to no-drug controls (n = 9 wells). Normality was detected by Shapiro-Wilk normality test. All data are expressed as mean ± SEM from ≥ 4 experiments. No significant change was detected at 15 (n = 5 wells) or 30 (n = 5 wells) min, nor 24 h (n = 4 wells), nor was there a significant change in the negative control gene *SP2* (n = 4). Students t-tests; $*p \leq 0.05$, $**p \leq 0.01$, $****p \leq 0.0001$.

4.1.2. *sAPPα Facilitates an Increase in Arc Protein Expression*

Previous research has shown a strong association between the increased expression of *Arc* mRNA and its rapid, activity- and experience-dependent translation (Steward et al., 1998; Steward et al., 2015). Interestingly, induction of Arc transcription leads to an increase in dendritic *Arc* mRNA, by active transport to sites of synaptic activity. In this sense Arc stands out from other immediate early genes which do not show this pattern of dendritic expression (Maroteaux et al., 2014), or such rapid activity-dependent transport (Steward et al., 1998). Therefore, examinations of both somatic and dendritic accumulation of Arc protein may give important insights into the regulation of plasticity by sAPPα. Thus, in order to examine Arc protein expression in our primary hippocampal neurons, we utilized immunocytochemistry and examined Arc expression in both somatic and dendritic compartments. Further, while evidence from our RT-qPCR data suggest a peak of *Arc* mRNA expression at 2 hours, this is reflected by past observations in behavioural learning paradigms (Hossaini et al., 2010; Lonergan et al., 2010; Soulé et al., 2012), and following synaptic activity (Chotiner et al., 2010) and BDNF treatment (El-Sayed et al., 2011). Therefore, we selected this timepoint for the examination of Arc protein following sAPPα treatment. Here, we found that sAPPα (1 nM, 120 min) increased both somatic (1.35 ± 0.46, $p \leq 0.0001$) and dendritic (1.92 ± 0.81, $p = 0.0003$) Arc protein expression relative to controls (Figure 4-2A,C,F,G). Similarly, 0.1 nM sAPPα significantly increased dendritic Arc expression (1.35 ± 0.05, $p = 0.0002$), but it did not significantly affect somatic Arc protein expression (1.15 ± 0.39, $p = 0.24$; Figure 4-2A-B,F,G).

While many plasticity-enhancing properties have been found of sAPPα, the β-secretase product, sAPPβ has shown few comparative properties. sAPPβ differs from sAPPα by 16 C-terminal amino acids, and has been described as 100-fold less effective in ameliorating excitotoxicity and attenuating glucose deprivation compared to sAPPα (Furukawa et al., 1996), is unable to protect against oligomeric Aβ-induced spine loss (Tackenberg and Nitsch, 2019), and does not enhance LTP (Mockett et al., 2019). Therefore, we next examined the effect of sAPPβ treatment (0.1 nM, 1 nM; 120 min) and found, in contrast to sAPPα, that neither 0.1 nM nor 1 nM sAPPβ significantly increased dendritic Arc protein (0.1 nM: 0.97 ± 0.49; 1 nM: 0.92 ± 0.45, $p \geq 0.99$; Figure 4-2A,D,F,G). Interestingly, while 0.1 nM sAPPβ did not affect Arc protein expression in the soma (0.91 ± 0.34, $p \geq 0.99$), 1 nM sAPPβ resulted in a small but significant decrease in somatic Arc expression (0.82 ± 0.38, $p = 0.03$; Figure 4-2A,E,F). Thus, the enhancement of Arc expression appears to be specific to sAPPα.

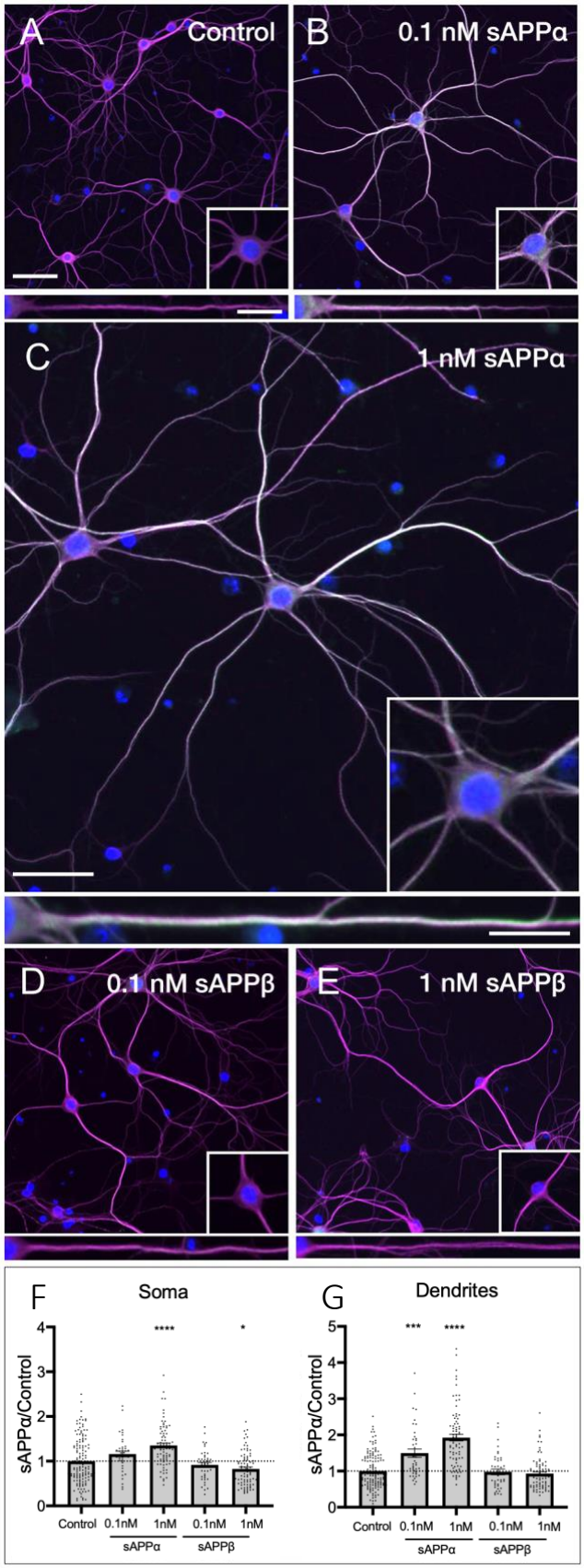


Figure 4-2 | sAPPα promotes Arc protein expression in a concentration-dependent manner. Representative images showing Arc protein levels in **A)** no drug control (*n* = 140 cells), **B)** 0.1 nM sAPPα (*n* = 40 cells), **C)** 1 nM sAPPα (*n* = 80 cells), **D)** 0.1 nM sAPPβ (*n* = 40 cells) and **E)** 1 nM sAPPβ-treated (*n* = 80 cells) primary hippocampal neurons. **F)** Average data showing 1 nM sAPPα promotes an increase, and 1 nM sAPPβ a modest decrease in Arc protein expression in the soma. **G)** Average data showing 0.1 and 1 nM sAPPα promotes an increase in dendritic Arc protein. All data are expressed as mean ± SEM from ≥ 4 experiments. Normality was detected by D'Agostino and Pearson omnibus normality tests, and significance was calculated using a Kruskal–Wallis one-way ANOVA with Dunn's multiple comparisons test on raw data. $*p = 0.0378$, $***p = 0.0002$, $****p \leq 0.0001$. Representative images show neuronal soma, dendrites (MAP2; ■), Arc protein (■), nuclei (DAPI; ■) and magnified somatic (inset, bottom right) and dendritic compartments. Scale bars = 50 µm (A-B,D-E), 100 µm (C); lower panels = 10 µm.

4.1.3. *sAPPα Promotes an Increase in Arc Protein Throughout Primary and Secondary Dendrites*

Following the observation that sAPPα (0.1 nM, 1 nM; 120 min) enhanced dendritic arc protein expression, we further aimed to validate these findings and extend this analysis, by assessing fluorescence intensity levels and distribution of Arc protein throughout the dendrites– a method previously employed Gumy et al., (2017). This assessment validated the finding that sAPPα (1 nM, 120 min) promotes an increase in Arc protein expression throughout the dendrites, as dendritic Arc expression was found to be significantly increased throughout the initial 50 µm (control: 40.06 ± 14.93; 0.1 nM sAPPα: 63.82 ± 20.1, $p \leq 0.0001$; 1 nM sAPPα: 62.04 ± 22.6, $p \leq 0.0001$; Figure 4-3), middle 50 µm (control: 32.43 ± 15.69; 0.1 nM sAPPα: 52.91 ± 25.57 $p = 0.0005$; 1 nM sAPPα: 52.89 ± 22.29, $p \leq 0.0001$) and final 50 µm segment of primary dendrites (control: 27.09 ± 11.58; 0.1 nM sAPPα; 43.72 ± 22.3, $p \leq 0.0001$; 1 nM sAPPα: 48.09 ± 24.17, $p = 0.0311$; Fig. 3A; Gilbride, 2016). Increased Arc expression in secondary dendrites was observed in the initial 25 µm (control: 32.54 ± 13.55; 0.1 nM sAPPα: 45.22 ± 19.61, $p = 0.01$; 1 nM sAPPα: 43.73 ± 21.08, $p = 0.01$) and middle 25 µm dendritic segment (control: 27.03 ± 14.08; 0.1 nM sAPPα: 37.64 ± 15.19, $p = 0.002$; 1 nM sAPPα: 36.63 ± 17.49, $p = 0.004$), however Arc expression was not significantly altered in the final 25 µm of secondary dendrites (control: 24.85 ± 12.25; 0.1 nM sAPPα: 31.99 ± 13.06, $p = 0.26$; 1 nM sAPPα: 30.06 ± 14.26, $p = 0.06$). While Arc expression has been previously shown to extend to distal dendrites (de Solis et al., 2017), the results observed here may indicate that signals regulating Arc expression or Arc protein itself fails to reach distal dendrites within the 2-hour window. Alternatively, this may indicate that processes of protein degradation may have already begun (Farris et al., 2014).

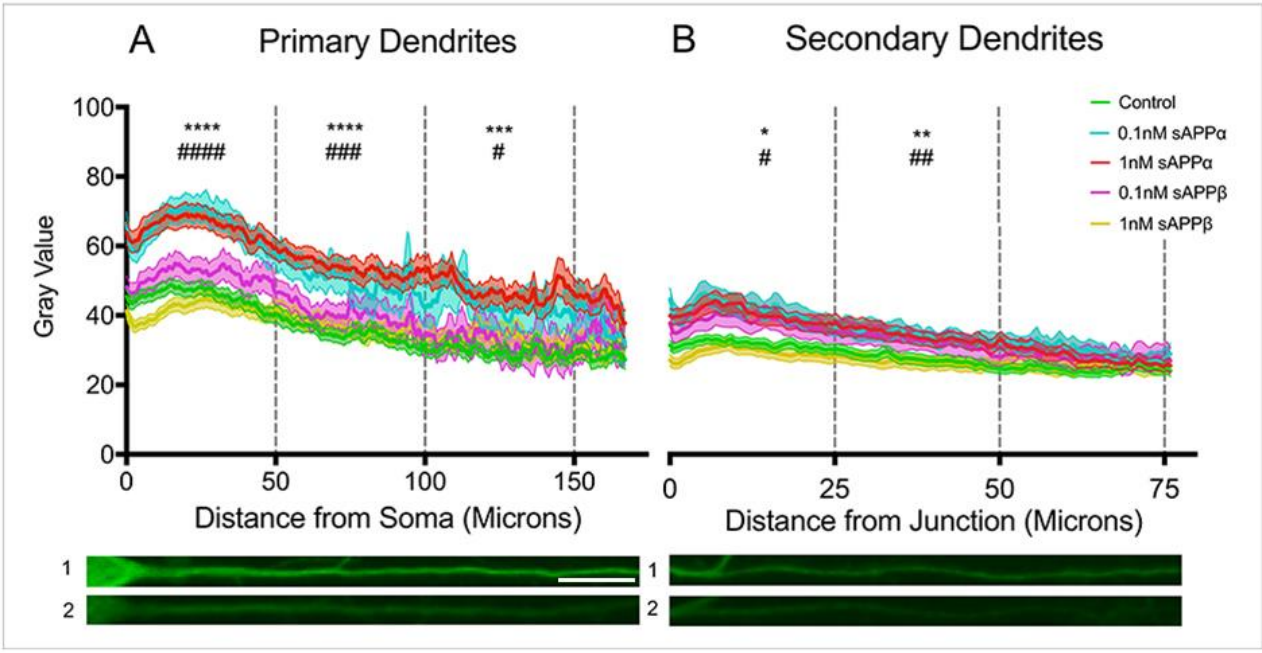


Figure 4-3 | Arc protein expression increases throughout primary and secondary dendrites. Average fluorescent intensity of Arc expression throughout (A) primary and (B) secondary dendrites (mean ± SEM, n = 40–141). Significance was determined by averaging dendritic gray value within each cell and is expressed per treatment group within (A) 50 and (B) 25-micron bins. Representative fluorescence images illustrate Arc protein expression (■) within primary (A1,2) and secondary (B1,2) dendrites in the presence of 1 nM sAPPα (A1, B1) or no drug (A2, B2). Asterisks (*) and hashes (#) indicate significance found between control and 1 nM and 0.1 nM sAPPα, respectively. Data are expressed a raw gray value (solid line) ± SEM (shaded area). All data are expressed as mean ± SEM from ≥ 4 experiments. Normality was detected by D'Agostino and Pearson omnibus normality tests, and significance was calculated using a Kruskal–Wallis one-way ANOVA with Dunn's multiple comparisons test on raw data. */#p = ≤ 0.05 **/##p ≤ 0.005 ***/###p = 0.0005, ****/####p ≤ 0.0001. Scale bar = 50 µm. Data was analysed with help from honours student Courteney Westlake.

4.1.4. *Arc Protein Expression is Transcription- and Translation-Dependent*

As shown by section 4.1.1. *sAPPα Facilitates an Increase in Arc and Zif268 mRNA Expression*, sAPPα (1 nM) enhances the expression of *Arc* mRNA in primary cortical neurons. Likewise, sAPPα (1 nM, 120 min) enhances the expression of Arc protein in both the soma and dendrites of cultured hippocampal neurons. To examine the relationship between these two events, we aimed to further examine whether the expression and localization of Arc would be otherwise affected by inhibition of both *de novo* transcription and translation in primary hippocampal neurons. Here, we found that sAPPα-induced Arc protein expression in both somatic (2.24 ± 1.830, $p \leq 0.0001$) and dendritic (1.99 ± 1.45, $p \leq 0.0001$) compartments was blocked by co-application of the transcription inhibitor actinomycin-D (Act-D; 10 µM; soma: 0.81 ± 0.68; dendrites: 0.95 ± 0.61, respectively; $p \leq 0.0001$; Figure 4-4A-C,E-F). Additionally, co-application of the translation inhibitor anisomycin (Aniso; 40 µM) also eliminated the effect (soma: 0.62 ± 0.30; dendrites: 0.84 ± 0.41, $p \leq 0.0001$; Figure 4-4A,B,D-F). These effects indicate that Arc protein expression at 2 hours is fully dependent on both *de novo* transcription and translation of mRNA and protein. These results reflect those observed by past literature examining the dependence of Arc transcription and translation during BDNF (Messaoudi et al., 2002; Zheng et al., 2009), dopamine (Fosnaugh et al., 1995), and nicotine (Schochet et al., 2005) treatment, as well as synaptic activity (Link et al., 1995; Lyford et al., 1995). Together, these findings add evidence to support the hypothesis that sAPPα promotes both Arc transcription and translation.

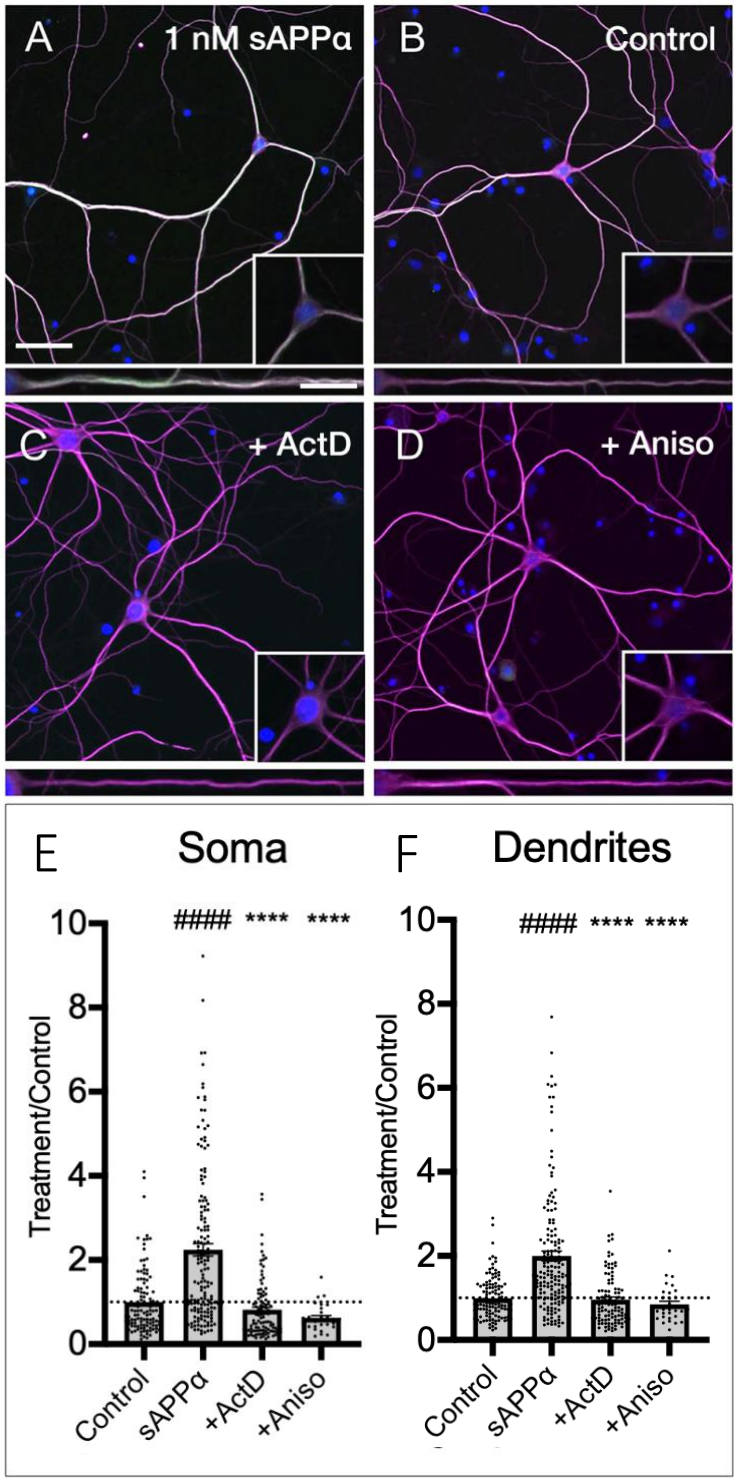


Figure 4-4 | Arc protein expression is prevented by inhibitors of transcription and translation. Representative images showing Arc protein levels in **A)** sAPPα (1 nM, 120 min, *n* = 157 cells) and **B)** no drug control (*n* = 115 cells) conditions. sAPPα-mediated Arc expression is inhibited with co-application of **C)** Actinomycin-D (*n* = 104 cells) or **D)** Anisomycin (*n* = 30 cells) in the **E)** soma and **F)** dendrites of hippocampal neurons. All data are expressed as mean ± SEM from ≥ 4 experiments. Normality was detected by D'Agostino and Pearson omnibus normality tests, and significance was calculated using a Kruskal–Wallis one-way ANOVA with Dunn's multiple comparisons test on raw data. Hashes (#) indicate significance between control and sAPPα-treated; asterisks (*) indicate significance between sAPPα- and inhibitor-treated; ****/####$p \leq 0.0001$. Representative images show neuronal soma, dendrites (MAP2; ■), Arc protein (■), nuclei (DAPI; ■) and magnified somatic (inset, bottom right) and dendritic compartments. Scale bars = 50 µm (A-D); lower panels = 10 µm.

4.1.5. *Arc Protein Expression is Dependent on CaMKII/MAPK/PKG Signalling*

Previous research has identified CaMKII, MAPK, and PKG as key downstream kinases in the regulation of cell surface GluA1 (Mockett et al., 2019), and the enhancement of synaptodendritic protein synthesis (Claasen et a., 2009). Here, we sought to determine if the translation or subcellular distribution of Arc protein utilizes similar processes. As well as this we aimed to extend our assay to examine protein kinases likewise linked to the regulation of Arc protein, synaptic plasticity, or protein synthesis, such as PKA, PKC, and mammalian target of rapamycin (mTOR; Waltereit et al., 2001; Boehm et al., 2006; Wang and Proud, 2006). Here, primary hippocampal cultures were incubated with sAPPα in the presence or absence of inhibitors for 2 hours with or without pre-treatment (refer Table 2-1. *Catalogue of Inhibitors and Antagonists used Throughout Immunocytochemical and Immunohistochemical Experiments*).

Accordingly, we found that sAPPα-mediated Arc protein expression was significantly reduced following inhibition of CaMKII in the soma (0.90 ± 0.48, $p \leq 0.0001$; Figure 4-5A,B,C,I). Arc protein expression in the soma was otherwise unaffected by inhibitors of MAPK (1.15 ± 0.46, $p = 0.47$; Figure 4-5A,B,D,I), PKG (1.48 ± 0.82, $p \leq 0.99$; Figure 4-5A,B,E,I), PKC (1.60 ± 0.83, $p \leq 0.99$; Figure 4-5A,B,F,I), PKA (1.5 ± 0.80, $p \geq 0.99$; Figure 4-5A,B,G,I) or mTOR (1.54 ± 0.71 $p \leq 0.99$; Figure 4-5A,B,H,I). Furthermore, dendritic Arc protein expression was significantly reduced through inhibition of CaMKII (0.72 ± 0.34, $p \leq 0.0001$; Figure 4-5A,B,C,J), MAPK (1.22 ± 0.80, $p = 0.0428$; Figure 4-5A,B,D,J), and PKG (0.97 ± 0.45, $p \leq 0.0001$; Figure 4-5A,B,E,J), however remained unaffected by inhibitors of PKA (1.57 ± 0.88, $p \geq 0.99$; Figure 4-5A,B,F,J), PKC (1.12 ± 0.51 $p = 0.0875$; Figure 4-5A,B,G,J), and mTOR (1.15 ± 0.58, $p = 0.0511$; Figure 4-5A,B,H,J). Given these results, and that of past research, it seems likely these processes may contribute to the transcription, translation, and localization of Arc in both the soma and dendrites following sAPPα treatment.

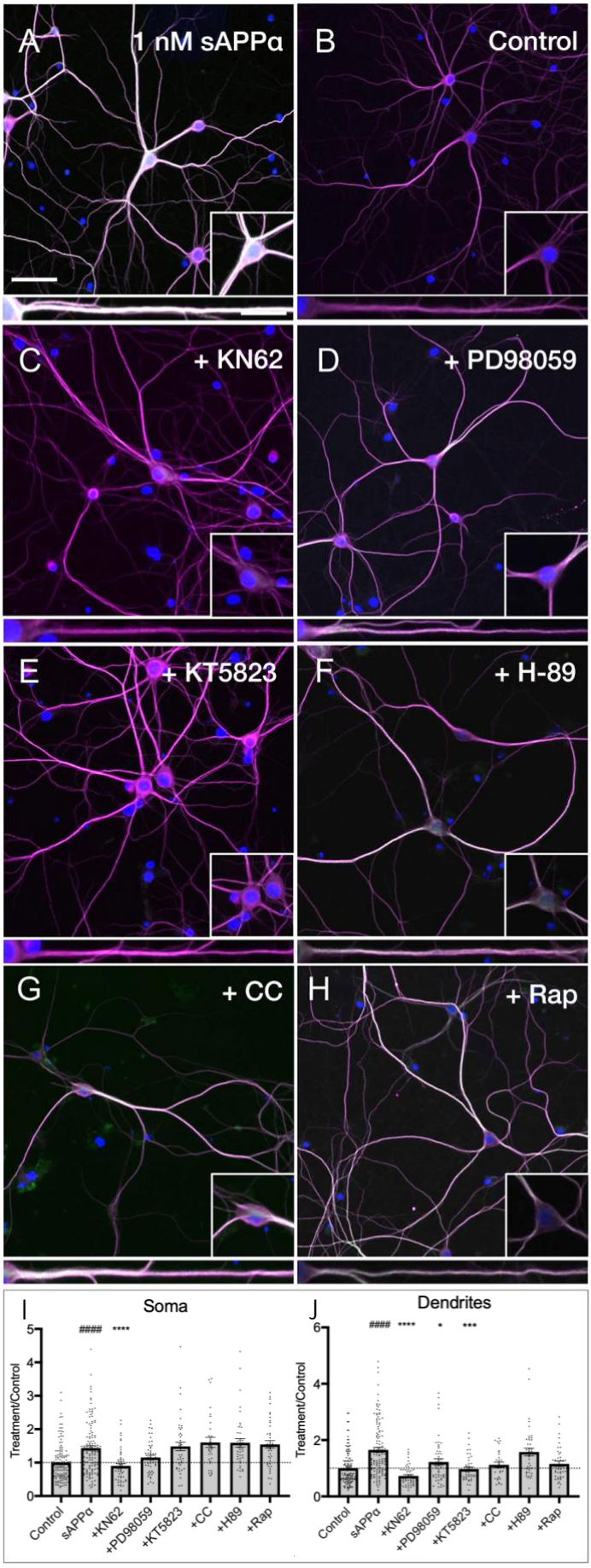


Figure 4-5 | Arc protein expression in response to sAPPα is affected by kinase inhibitors. Representative images showing **A)** sAPPα (1 nM, 120 min, *n* = 125 cells) promotes an increase in the expression of Arc protein in cultured neurons relative to **B)** no drug controls (*n* = 134 cells). Cells were simultaneously treated with **C)** KN62 (*n* = 51 cells), **D)** PD98059 (*n* = 51 cells), and E) KT5823 (*n* = 42 cells), **F)** H-89 dihydrochloride (H89; *n* = 40 cells), **G)** Chelerythrine chloride (CC; *n* = 30 cells), or **H)** Rapamycin (Rap; *n* = 40 cells) and Arc levels in the **I)** soma and **J)** dendrites was measured. All data are expressed as mean ± SEM from ≥ 3 experiments. Normality was detected by D'Agostino and Pearson omnibus normality tests, and significance was calculated using a Kruskal–Wallis one-way ANOVA with Dunn's multiple comparisons test on raw data. Hashes (#) indicate significance between control and sAPPα-treated; asterisks (*) indicate significance between sAPPα- and inhibitor-treated; **p* = 0.0428, ****p* = 0.001, ####/*****p* ≤ 0.0001. Representative images show neuronal soma, dendrites (MAP2; ■), Arc protein (■), nuclei (DAPI; ■) and magnified somatic (inset, bottom right) and dendritic compartments. Scale bars = 50 µm (A-D); lower panels = 10 µm.

4.1.6. *Dendritic Arc Protein Expression is Dependent on Activation of NMDA- and α7nACh Receptors*

Despite many studies, the cell surface receptor or receptors which transduce signals downstream of sAPPα are yet to be conclusively identified, though candidates have emerged. These include NMDAR, α7nAchR, sorting-related receptor with A-type repeats (SORLA), gamma aminobutyric acid receptors ($GABA_B$), and membrane-bound APP itself (Rice et al., 2017; Hartl et al., 2013; Milosch et al., 2014; Richter et al., 2018; Mockett et al., 2019). Here, we pharmacologically inhibited likely candidates mediating sAPPα's plasticity-promoting effects and observed the effect on Arc protein levels (Figure 4-6). We found that application of antagonists targeting $GABA_B$ (CPG55845; 50 µM), TrkB (ANA-12; 100 µM), or mGluRI/II receptors (MCPG; 500 µM) had no significant effect on dendritic Arc protein expression (Figure 4-6A,B,I) following sAPPα treatment (1 nM, 120 min; CPG55845: 2.79 ± 1.08, $p \geq 0.99$, Figure 4-6C,I; ANA-12: 2.78 ± 1.29, $p \geq 0.99$, Figure 4-6D,I; MCPG: 2.29 ± 1.27, $p \geq 0.99$, Figure 4-6E,I). However, Arc protein expression was significantly reduced following antagonism of NMDA receptors by APV (50 µM; 1.67 ± 0.78, $p = 0.01$; Figure 4-6F,I) and α7nAch receptors with α-bungarotoxin (αBGT, 10 nM; 1.61 ± 1.18, $p = 0.0138$; Figure 4-6G,I). Combined antagonism of NMDA and α7nAch receptors completely abolished sAPPα-mediated Arc expression (0.78 ± 0.49, $p \leq 0.0001$; Figure 4-6H,I). Previous work has established a coupling between NMDA- and α7nACh Receptors (Li et al., 2012; Li et al., 2013), promoting a synergism of activity (Aramakis et al., 1998; Bali et al., 2017; Bali et al., 2019). Due to this, these results may suggest a novel mechanism whereby synergistic action between NMDA and α7nAch receptors govern an enhancement in sAPPα-mediated Arc protein expression.

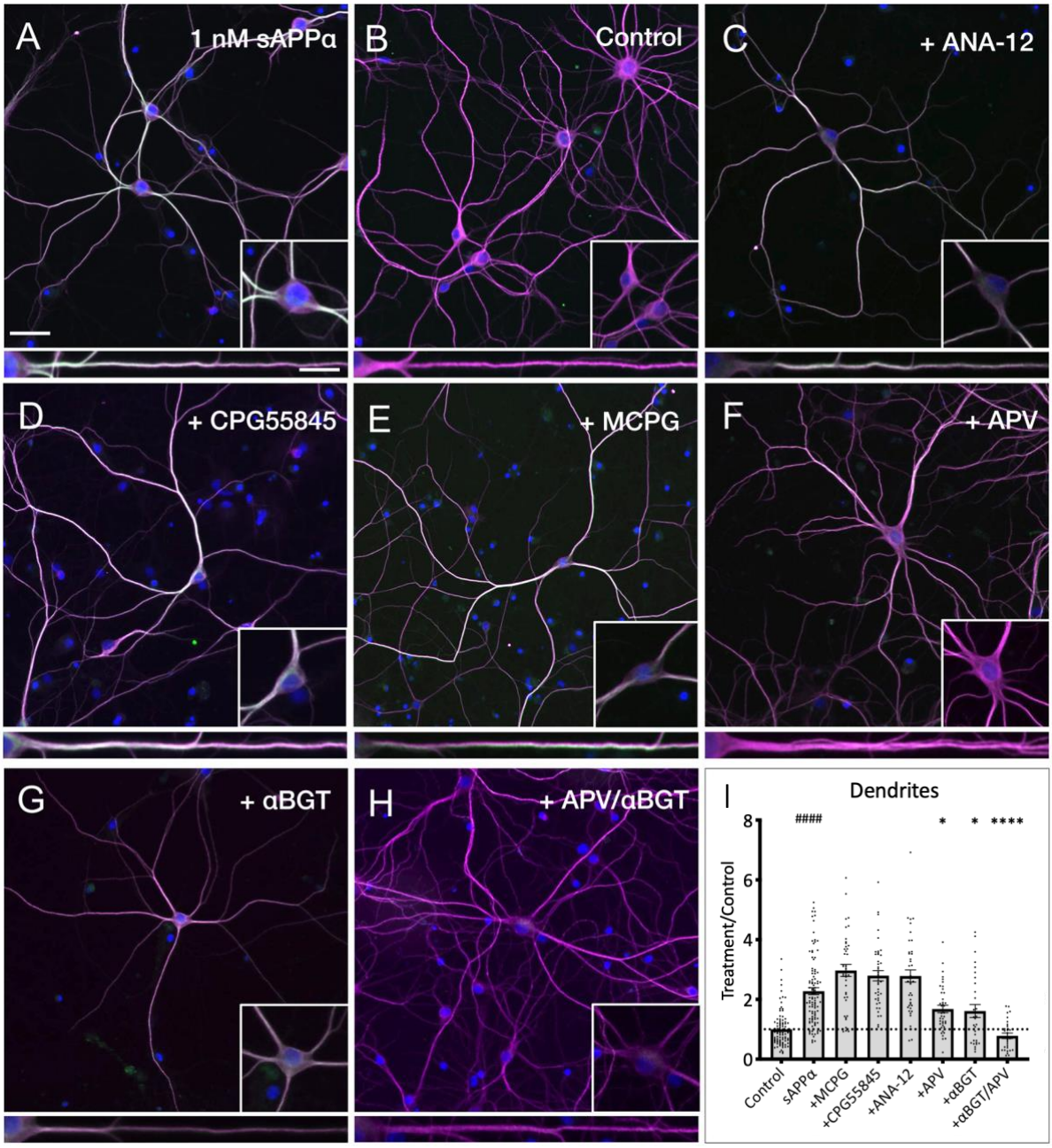


Figure 4-6 | Arc protein expression is dependent on NMDA and α7nAch receptors. Representative images showing A) sAPPα (1 nM, 120 min, *n* = 80 cells) promotes and increase in the expression of Arc protein in cultured hippocampal neurons relative to B) no drug controls (*n* = 80 cells). Co-incubation of sAPPα with C) ANA-12 (*n* = 40 cells), D) CPG55845 (*n* = 40 cells), and E) MCPG (*n* = 40 cells) had no effect on sAPPα-induced Arc expression. Co-incubation with F) APV (*n* = 40 cells), or G) αBGT (*n* = 30 cells) significantly reduced Arc protein expression, while co-incubation with both H) APV and αBGT (*n* = 30 cells) fully eliminated this effect in the I) dendrites. Outliers were removed from each experiment prior to amalgamation using Grubb's tests, and normality was detected by D'Agostino and Pearson omnibus normality tests. All data are expressed as mean ± SEM from ≥ 3 experiments. Significance was calculated using a Kruskal–Wallis one-way ANOVA with Dunn's multiple comparisons test on raw data. Hashes (#) indicate significance between control and sAPPα treated; asterisks (*) indicate significance between sAPPα- and antagonist-treated; $^{*}p \leq 0.05$, $^{\#\#\#\#}p = 0.0001$, $^{****}p \leq 0.0001$. Representative images show neuronal soma, dendrites (MAP2; ■), Arc protein (■), nuclei (DAPI; ■) and magnified somatic (inset, bottom right) and dendritic compartments. Scale bars = 50 µm (A-D); lower panels = 10 µm.

4.2. *sAPPα Increases CREB Phosphorylation and Arc Protein in Acute Hippocampal Slices*

So far, this thesis has described sAPPα's ability to enhance Arc protein expression *in vitro,* in a manner dependent on CaMKII, MAPK, PKG signalling and activation of NMDAR and α7nAchRs. Previous work has shown that the enhancement of LTP and cell surface GluA1 by sAPPα is likewise dependent on these processes (Richter et al., 2018; Mockett et al., 2019). Additionally, Arc expression throughout hippocampal areas CA1 (Penke et al., 2011; Jakkamsetti et al., 2013; Gao et al., 2018), CA3 (Rosi et al., 2005; Chawla et al., 2018a; Chawla et al., 2018b; Hudgins and Otto, 2019), and dentate gyrus (Messaoudi et al., 2007; Ramirez-Amaya et al., 2013; Kuipers et al., 2016) have been shown to be important for many functional outcomes of memory. Thus, the question remains whether sAPPα promotes an equally significant increase in Arc protein expression in acute hippocampal slices, as observed in primary hippocampal neurons.

Here, we aimed to extend our analyses to examine Arc expression in acute hippocampal slices, as well as further validate the relationship between NMDAR and α7nAchR activation following sAPPα treatment, by inhibition of these receptors and examination of phosphorylated Ca^{2+}/cAMP-response element-binding protein (pCREB), a signalling event strongly associated with NDMAR activity (Xia et al., 1996; Sala et al., 2000; Valera et al., 2008; de Oliveira Coelho et al., 2013; Zhou et al., 2013). We hypothesised that in addition to enhancing Arc protein expression, sAPPα (1 nM, 15 min) would enhance the expression of pCREB in the cell body layer of area CA1 and the dentate gyrus of the hippocampus. Within area CA1 of acute hippocampal slices (Figure 4-7A), we found that sAPPα (1 nM, 15 min) significantly increased pCREB levels (2.08 ± 0.60, p = 0.01; Figure 4-7B,C,G), supporting a role for NDMAR activity. Further, we found that sAPPα (1 nM, 120 min) significantly increased Arc expression (1.55 ± 0.22, p = 0.02; Figure 4-7D,E,H) and that this effect was attenuated by co-incubation with NMDA and α7nAch antagonists APV (50 μM) and αBGT (10 nM; 1.08 ± 0.18, p = 0.042; Figure 4-7F,H), validating the observations from primary hippocampal cultures.

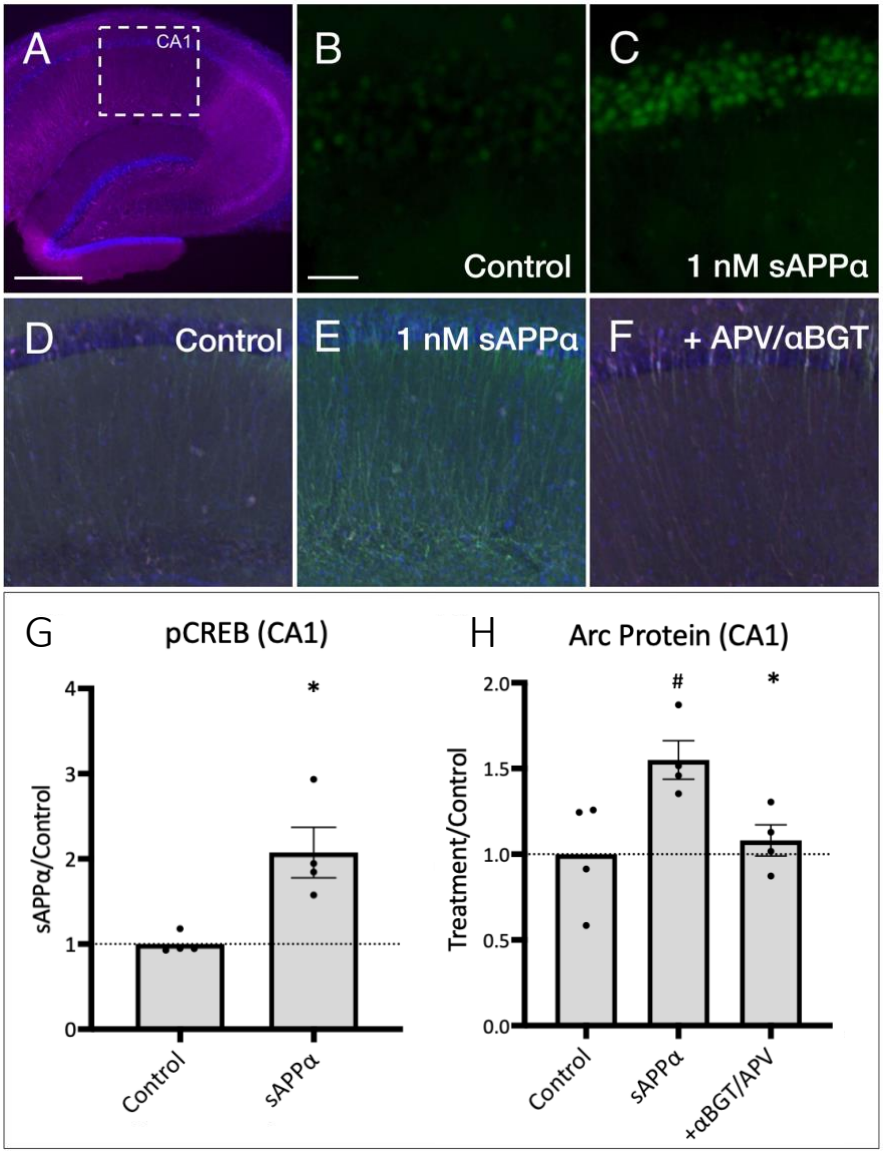


Figure 4-7 | sAPPα increases CREB phosphorylation and Arc protein in acute hippocampal slices. A) A representative transverse section of an acute hippocampal slice, with a sub-region of area CA1, used for quantitative analysis, outlined by a white dotted box (MAP2 ( ); nuclei: DAPI ( ); imaged at 4x magnification; scale bar = (500 µm). Relative to B) no drug controls (*n* = 3 rats, 4 slices), C) incubation of slices with sAPPα (1 nM, 15 min, *n* = 2 rats, 4 slices) increased pCREB (; imaged at 20x magnification; scale bar = 100 µm) in the PCL of CA1. Relative to D) no drug controls (*n* = 3 rats, 4 slices), E) incubation of slices with sAPPα (1 nM, 2 hr, *n* = 3 rats, 4 slices) significantly increased Arc protein expression (; imaged at 4x magnification) in area CA1. Co-incubation of sAPPα with F) APV and αBGT (*n* = 3 rats, 4 slices) attenuated this effect. Normality was detected by Shapiro–Wilk normality tests. All data are expressed as mean ± SEM. Significance of pCREB (G) and (H) Arc protein expression was calculated by G) student's t-test, and H) one-way ANOVA with Šidák's multiple comparisons test on raw data. Hashes (#) indicate significance between control and sAPPα-treated; asterisks (*) indicate significance between sAPPα- and antagonist-treated; $^{\#}p \leq 0.05$, $^{*}p = 0.04$. PCL = pyramidal cell layer.

4.2.1. *Trend in CREB Phosphorylation and Arc Protein Expression in the Dentate Gyrus of Acute Hippocampal Slices*

Given the involvement of both pCREB and Arc protein expression in synaptic activity in the dentate gyrus (Schulz et al., 1999; Bramham et al., 2010), we further sought to expand our analysis to examine expression of pCREB and Arc in the hilus of the dentate gyrus of acute hippocampal slices. Converse to results observed in area CA1, we found a small but non-significant increase in pCREB in the hilus of the dentate gyrus (1.17 ± 0.058, p = 0.0858; Figure 4-8A-C). Likewise, Arc protein in the DG showed a small increase in expression (sAPPα: 1.15 ± 0.18, p = 0.6953; APV/αBGT: 0.98 ± 0.13, p = 0.6610; Figure 4-8H) but did not reach significance, reflecting a similar trend to pCREB expression.

Interestingly, while many publications have described the induction of Arc protein within the dentate gyrus following spatial exploration (Ramirez-Amaya et al., 2013), LTP (Messaoudi et al., 2007), and BDNF treatment (Kuipers et al., 2016), these results may be due to the sensitivity of our analysis. The relatively small sample size, as well as the large variation in control groups examining both pCREB (coefficient of variation (CV): control: 27.26%, sAPPα: 4.96%) and Arc (CV: control: 33.71%, sAPPα: 16.21%, APV/αBGT: 13.28%) protein expression may have contributed to the observed lack of effect within these analyses. Alternatively, we have shown that this effect requires the activation of both α7nAChR and NMDAR. While the presence of NMDAR in the dentate gyrus is unquestioned (Bernabeu and Sharp, 2000; Dalby and Mody, 2003; Wright and Jackson, 2014), evidence suggests an absence of α7nAChR-containing excitatory granule cells in these neurons (Frazier et al., 2003). While synergistic activation of α7nAChR and NMDAR is required for Arc expression (see section 4.1.6. *Dendritic Arc Protein Expression is Dependent on Activation of NMDA- and α7nACh Receptors*), it may be expected that sAPPα would not induce the full complement of Arc protein expression in this paradigm. The small but non-significant increase in pCREB (Figure 4-8A-C) and Arc protein (Figure 4-8H) in the dentate gyrus may reflect the NMDAR component of this.

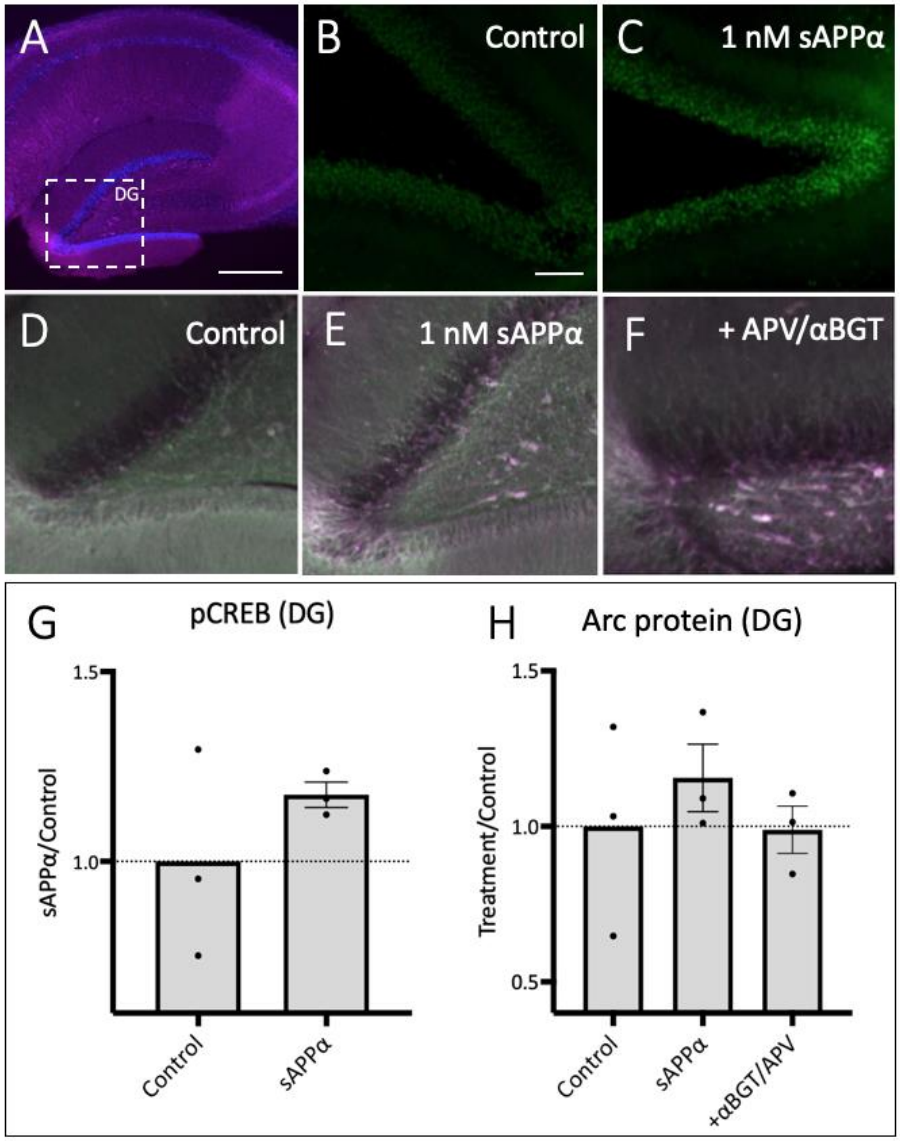


Figure 4-8 | sAPPα does not significantly increase pCREB or Arc protein in the hilus of the dentate gyrus in acute hippocampal slices. Representative transverse sections of A) acute hippocampal slices in the hilus of the dentate gyrus (MAP2 ( ); nuclei: DAPI (; imaged at 4x magnification). Relative to B) no drug controls (*n* = 2 rats, 3 slices), incubation of slices with C) sAPPα (1 nM, 15 min, *n* = 2 rats, 3 slices) did not significantly increase pCREB relative to controls, as assessed by student's t-test (; imaged at 20x magnification). Relative to D) no drug controls (*n* = 2 rats, 3 slices), E) incubation of slices with sAPPα (1 nM, 120 min, n = 2 rats, 3 slices) did not significantly increase Arc protein expression (; imaged at 20x magnification) in the DG. Similarly, co-incubation of sAPPα with F) APV and αBGT (*n* = 2 rats, 4 slices) did not affect Arc protein expression. All data are expressed relative to control, as mean ± SEM. Normality was detected by Shapiro–Wilk normality tests, and significance was assessed by H) student's t-test and H) one-way ANOVA with Šidák's multiple comparisons test on raw data. Scale bars = 500 µm (A), 100 µm (B-D).

4.2.2. *Increases in Arc Protein is Specific to Area CA1 and Does Not Occur in Area CA3*

Following the observation that Arc protein expression increases in area CA1 in response to sAPPα-treatment, we further examined Arc protein expression in area CA3 of acute hippocampal slices. Here, we found no significant increase in Arc protein relative to control (sAPPα: 1.55 ± 0.22, p = 0.6343; APV/αBGT: 1.080 ± 0.18, p = 0.9630; Figure 4-9).

While these data may reflect the relatively small sample size, these observations may also arise through differences in the anatomical and molecular structure of synapses terminating in area CA3, compared to those in CA1 and the DG. LTP at CA3 mossy fibre synapses occurs through primarily presynaptic means, independent of postsynaptic NMDAR activation (Harris and Cotman, 1986; Lysetskiy et al., 2005), postsynaptic Ca^{2+} influx (Zalutsky and Nicoll, 1990), and may not involve an AMPAR component (Bliss et al., 2003). Interestingly, α7nAChR may play a unique role in enhancing synaptic activity at these synapses through their enhancement of presynaptic Ca^{2+} currents and neurotransmitter release (Gray et al., 1996; Radcliffe et al., 1999; Sharma and Vijayaraghavan, 2003; Sharma et al., 2008; Grybko et al., 2010). Regardless, it appears sAPPα may enhance Arc expression and synaptic plasticity independent from these mechanisms.

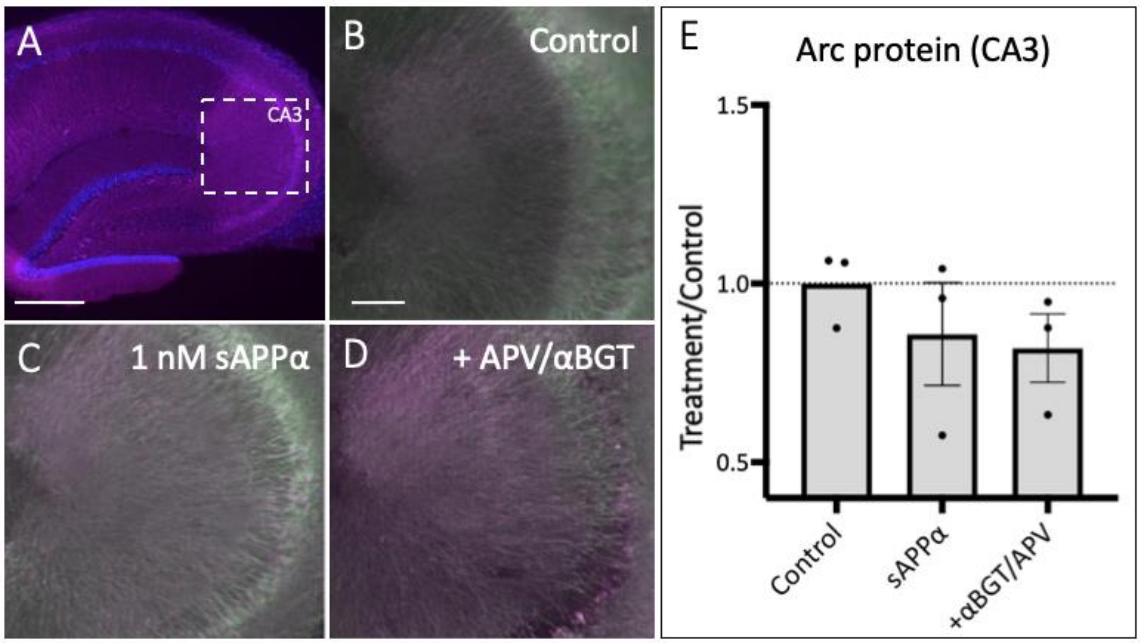


Figure 4-9| sAPPα does not increase Arc protein in area CA3. A) Representative transverse sections of acute hippocampal slices in area CA3 (MAP2 ( ); nuclei: DAPI (; imaged at 4x magnification). In area CA3, relative to B) no drug controls (*n* = 2 rats, 3 slices), incubation of slices with C) sAPPα (1 nM, 120 min, *n* = 2 rats, 3 slices) did not significantly increase Arc protein relative to controls, similarly D) APV and αBGT significantly alter Arc protein expression. Normality was detected by Shapiro–Wilk normality tests. E) Data are expressed relative to control, as mean ± SEM. Significance was assessed by one-way ANOVA with Šidák's multiple comparisons test on raw data. Representative images show MAP2 ( ), and Arc protein ( ), imaged at 20x magnification. Scale bars = 500 µm (A), 100 µm (B-D).

4.3. *Summary*

The work described in this chapter aimed to characterise the expression of the IEG Arc in cultured hippocampal neurons, which was enhanced following incubation with sAPPα (1 nM, 120 min). This expression was paired with an increase in *Arc* and *Zif268* mRNA in a time dependent manner. In line with published research examining the role of sAPPα in protein synthesis and trafficking of GluA1, the expression of Arc protein in both somatic and dendritic neuronal compartments was found to be differentially regulated following inhibition of CaMKII, MAPK, and PKG. Further, we have defined a novel role for the synergistic activity of NMDA- and α7nACh receptors, in mediating this effect. Replicating these experiments in area CA1 of acute hippocampal slices validated the dependency on NMDA- and α7nACh receptors in the expression of Arc protein, and was found to be coupled to the expression of pCREB, indicating a necessary role of NMDAR activation as a downstream signalling event. Interestingly, both the increase in pCREB and Arc protein was found to be specific to area CA1, as no significant change was present in area CA3 nor the hilus of the dentate gyrus. Importantly, these experiments are limited due to the lack of inhibitor or antagonist-only controls as well as the use of only one inhibitor per kinase. Previous work has shown that inhibitors of CaMKII, PKG, MAPK and protein synthesis, as well as the NDMAR antagonist APV have little to no effect on glutamate receptor trafficking and phosphorylation (Mockett et al., 2019), while both NMDAR and α7nACh receptors have been shown to provide little to spontaneous synaptic currents in culture (Hefft et al., 1999; Hmaied et al., 2002). However, these experiments cannot rule out any drug-only effects on baseline kinase or receptor activity nor draw conclusions on the specificity of the kinases targeted.

Regardless, these results support the hypothesis that sAPPα enhances both the synthesis and the dendritic expression of Arc protein. While this may indicate a role of Arc in mediating the expression of the sAPPα-mediated enhancement of LTP, further experiments are required to link the function of Arc to the regulation of plasticity following sAPPα treatment.

Chapter 5

5.1. *The Role of Calcium-Permeable AMPAR (CP-AMPAR) in sAPPα-Mediated Enhancement of LTP*

Previous work has shown that sAPPα (1 nM, 30 min) enhances both the induction and persistence of LTP, and increases cell surface levels of GluA1-, but not GluA2-containing AMPAR, in a protein synthesis-dependent manner following mild TBS (Mockett et al., 2019). This specific increase of GluA1 on the cell surface suggests a role for GluA1-containing homomeric AMPAR in regulating sAPPα-enhanced LTP. This chapter aimed to examine the hypothesis that sAPPα (1 nM, 30 min) may enhance synaptic transmission by enhancing the expression of GluA1-containing AMPAR at the synapse.

Here, we sought to test this hypothesis by taking advantage of the susceptibility of GluA1 homomeric CP-AMPAR to blockade by polyamine-containing inhibitors such as N,N,N-trimethyl-5-[(tricyclo[3.3.1.13,7]dec-1-ylmethyl)amino]-1-pentanaminium bromide hydrobromide (IEM-1460; Schlesinger et al., 2005). IEM-1460 contains a positively-charged polyamine tail and hydrophobic adamantane head, blocking the ion channel of GluA2-lacking AMPAR in a voltage-dependent manner (Twomey et al., 2018), with a relatively high affinity, reversibility (Samoilova et al., 1999) and specificity to GluA2-lacking Ca^{2+}-permeable AMPAR (Samoilova et al., 1999). Experiments utilized an LTP-inducing stimulation protocol which enhances synaptic transmission, but is non-saturating (half-maximal train of standard TBS: 5 trains of 5 pulses at 100 Hz delivered at 200 ms intervals) as per Mockett et al, (2019).

5.1.1. *CP-AMPAR Contribute a Small Fraction of Basal Synaptic Transmission*

Importantly, previous research has shown that only 8% of total AMPA receptor complexes exist as homomeric GluA1 in CA1/CA2 hippocampal pyramidal neurons, under basal conditions (Wenthold et al., 1996). Due to this, previous observations have shown that administration of CP-AMPAR antagonists has little-to-no effect on basal CA1 synaptic transmission (Plant et al., 2006; Sutton et al., 2006; Adesnik and Nicoll, 2007; Park et al., 2016), indicating a lack of synaptic CP-AMPARs, or levels below the sensitivity of detection.

To first examine the role of CP-AMPAR in basal synaptic transmission in our preparation, acute hippocampal slices (400 µm) were prepared from young adult male Sprague-Dawley rats (4-6 weeks), and transferred to a recording chamber (refer section 2.5.1. *Acute Hippocampal Slice Preparation*). Following the establishment of a stable baseline for 20 minutes (Figure 5-1A), IEM-1460 (100 µM, 50 min) was applied to slices and baseline responses were measured. Here we found that application of IEM-1460 (20 min) resulted in a modest, but significant reduction in baseline responses (94.62 ± 3.82% of baseline, p = 0.0002, n = 12; Figure 5-1B). An additional 30 minutes of IEM-1460 further reduced baseline transmission (average decrease of 8.3%) relative to initial baseline values, however this did not reach significance (91.70 ± 6.71% of baseline, p = 0.0573; Figure 5-1B). While previous publications have utilized IEM-1460 to investigate the role of CP-AMPAR in various applications across a range of concentrations (30-200 µM), those utilizing IEM-1460 at similar concentrations to the current study did not report any significant effects on baseline transmission. This may result from many factors including animal species, *in vivo* versus *ex vivo*, differences in slice preparations, and importantly, the age of the animal used (Gray et al., 2007; Asrar et al., 2009; Sanderson et al., 2016; Suyama et al., 2017).

We further aimed to examine whether treatment of hippocampal slices with sAPPα would affect baseline responses. Here we found that sAPPα (1 nM, 30 min) did not significantly alter baseline responses from initial values (99.73 ± 5.35%, p = 0.4070). Importantly, these results indicate that sAPPα treatment alone does not involve the direct potentiation of synaptic transmission, indicating that translocation of AMPAR into the synapse may require additional stimulation.

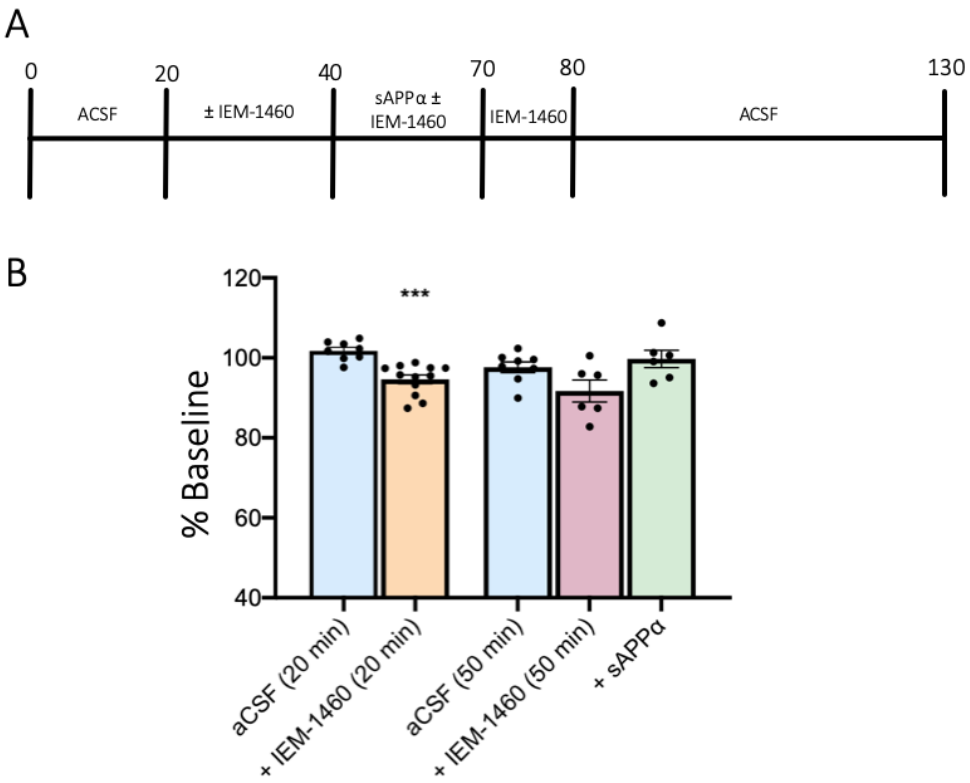


Figure 5-1| Reduction in basal synaptic transmission following CP-AMPAR blockade. A) Representative schematic of the experimental design, values represent time points (min) of key events during the recording period. B) Data showing averaged fEPSP measurements as percentage from initial baseline values following 20 minutes of IEM-1460 (●) and an equivalent 20 minutes of aCSF (20 min aCSF; ●), or an additional 30 minutes IEM-1460 (●), and 30 minutes of sAPPα treatment (●), and an equivalent 30 minutes of aCSF (50 min aCSF; ●) in control (n = 8 slices), IEM-1460 treated (n = 12 slices), sAPPα-treated (n = 6 slices) conditions from 6-8 animals. Data from multiple experiments following 20 min of IEM-1460 treatment were pooled. All data were measured as an average of the final 10 minutes of baseline stimulations at each interval, relative to percent of baseline in the presence of aCSF only. Normality was determined by Shapiro-Wilk normality test, and significance was calculated using an Student's t-test. Asterisks (*) indicate significance between antagonist-treated and control, ***$p \leq 0.001$.

5.1.2. *CP-AMPAR Do Not Contribute to LTP Following a Mild Theta-Burst Stimulation Protocol*

In order to establish whether CP-AMPAR are involved in the enhancement of LTP following sAPPα treatment and mild TBS, the contribution of CP-AMPAR to LTP elicited by our mild TBS protocol alone needed to be established. As previously described (refer section 2.5.1. *Acute Hippocampal Slice Preparation*), hippocampal slices were prepared from young adult male Sprague-Dawley rats (4-6 weeks), maintained in an incubation chamber for 120 minutes, and transferred to a recording chamber. Following the establishment of a stable baseline for 20 minutes, acute hippocampal slices were treated with the CP-AMPAR antagonist IEM-1460 (100 µM; 50 min) to ensure extrasynaptic, perisynaptic and trafficked AMPAR were inhibited prior to TBS. Perfusion of IEM-1460 continued 10 minutes post-TBS after which the recording chamber was switched back to standard aCSF for the remainder of the recording. Here we have examined the enhancement of synaptic transmission as an average of the first (early LTP) and final (late LTP) 10 minutes following TBS.

As shown by Figure 5-2., application of IEM-1460 (100 µM, 50 min) had no significant effect on the magnitude of either early (control: 52.10 ± 17.53% of baseline, n = 8; IEM-1460: 59.66 ± 17.00% of baseline, n = 6, p = 0.9399) or late (control: 32.79 ± 17.39% of baseline; IEM-1460: 28.44 ± 10.31% of baseline, p = 0.9873) potentiation following TBS, supporting past research (Adesnik and Nicoll, 2007; Gray et al., 2007; Asrar et al., 2009). Thus, we can conclude that CP-AMPARs do not normally play a significant role in the potentiation of synaptic transmission following a mild TBS protocol, and therefore conclusions drawn following treatment of slices with sAPPα may be attributed to mechanisms employed by sAPPα.

5.1.3. *CP-AMPAR Contribute to the Initial Enhancement of sAPPα-LTP*

Here, we aimed to first replicate previous observations that sAPPα (1 nM, 30 min) enhances both E- and L-LTP following TBS (Mockett et al., 2019), and further examine the role of CP-AMPAR in mediating this effect. LTP was elicited in area CA1 of hippocampal slices in the presence or absence of sAPPα (1 nM) 30 minutes before application of TBS. To examine the role of CP-AMPAR in sAPPα-mediated LTP, slices were pre-treated with IEM-1460 (100 μM) for 20 minutes, in addition to co-treatment with sAPPα (1 nM) for 30 minutes, and 10 minutes post-TBS stimulation in the absence of sAPPα.

As shown in Figure 5-2, relative to control slices, pre-incubation with sAPPα (1 nM, 30 min) enhanced both early (control: 52.1 ± 17.53%, n = 8; sAPPα: 126.2 ± 33.20% of baseline, n = 6, $p \leq$ 0.0001; Figure 5-2C,D) and late (control: 32.79 ± 17.39%; sAPPα: 90.33 ± 15.91% of baseline, p = 0.0014; Figure 5-2C,E) phase LTP, confirming previously observed enhancements described by Mockett et al., (2019). Co-application of IEM-1460 with sAPPα significantly inhibited this sAPPα-mediated early potentiation relative to sAPPα-treatment alone (84.19 ± 28.76% of baseline, n = 6, p = 0.0334; Figure 5-2C,D). Wash-out of IEM-1460 resulted in the recovery of potentiation, returning potentiation to a level comparable to sAPPα-induced enhancement (69.39 ± 38.22% of baseline, p = 0.4784; Figure 5-2C,E), which was significantly different from control slices (p = 0.0438). These results indicate that the initial potentiation of sAPPα-enhanced LTP is due to the rapid incorporation of functional CP-AMPAR at the synapse. The return of potentiation following washout may indicate that these AMPAR persist at the synapse throughout the recording duration, however these experiments do not rule out the possibility of potentiation mediated by GluA2-containing AMPAR.

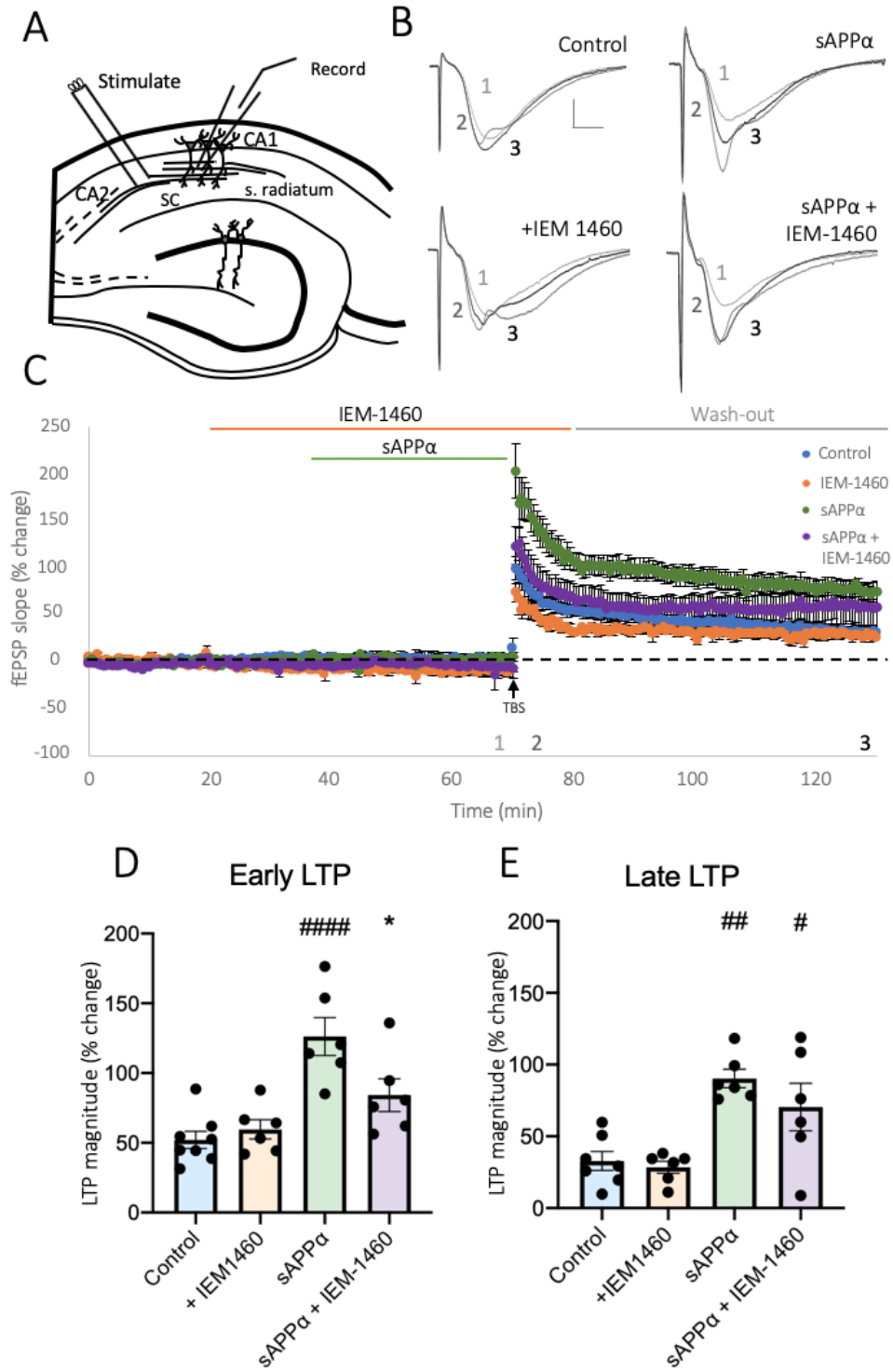


Figure 5-2 | Early phase sAPPα-enhanced LTP is dependent on activation of CP-AMPAR. A) Representative schematic of transverse hippocampal slices showing positioning of stimulation and recording electrodes in area CA1. B) Representative field excitatory postsynaptic potential (fEPSP) traces taken at the end of baseline recording (1), upon TBS (2), and 60 min post-TBS (3). Scale bar = 1 mV, 5 s. C) Average traces of control slices, receiving only a mild TBS (5 bursts at 5 Hz, 5 pulses at 100 Hz/burst; ●, *n* = 8 slices), IEM-1460 (100 µM; ●, *n* = 6 slices), sAPPα (1 nM, ●, *n* = 6 slices) and sAPPα + IEM-1460-treated (●, *n* = 6 slices) conditions. All conditions are normalised to the average of baseline 10 minutes before application of IEM-1460, and all data are presented as mean % change ± SEM. Data shows summary histograms for D) early and E) late potentiation following TBS. Data are averaged from the first (D) and final (E) 10 minutes potentiation post-TBS for each experimental group relative to baseline, averaged 10 minutes before TBS. **p* = 0.0334, $^{\#}p$ = 0.0438, $^{\#\#}p$ = 0.0014, $^{\#\#\#\#}p \leq 0.0001$. Normality was detected by Shapiro-Wilk normality test. Significance was assessed by one-way ANOVA with Tukey's multiple comparisons tests. Hashes (#) indicate significance between control and sAPPα-treated; asterisks (*) indicate significance between sAPPα- and antagonist-treated. SC = Schaffer collaterals, s. radiatum = Stratum radiatum.

5.2. *Summary*

The results described in this chapter aimed to examine the contribution of CP-AMPAR to mild TBS-induced LTP, as well as LTP enhanced by sAPPα treatment (1 nM, 30 min). Additionally, we aimed to assess the effect of the CP-AMPAR antagonist IEM-1460 on baseline responses, before the induction of LTP. Here, we found a small, but significant effect of IEM-1460 on baseline synaptic transmission in our acute hippocampal slices. Further, we have provided evidence for a dependency of CP-AMPAR activity during the induction of sAPPα-primed LTP. Conversely, LTP induced by mild TBS alone was not dependent on the expression of these AMPARs. These results support the hypothesis that sAPPα primes LTP through the expression of CP-AMPAR. Importantly, sAPPα treatment alone does not appear to enhance the synaptic expression of these AMPAR as baseline synaptic transmission remined unaltered. Thus, these results may indicate that that sAPPα sequesters CP-AMPAR at perisynaptic or extrasynaptic domains prior to the induction of LTP. Due to past observations that sAPPα (1 nM, 30 min) enhances trafficking of GluA1-containing AMPAR to the cell surface (Mockett et al., 2019), it is possible that the origin of these AMPAR may be from internal stores, but lateral diffusion from extrasynaptic sites cannot be ruled out. Regardless, these results describe a key role of CP-AMPAR in the induction of sAPPα-mediated LTP.

Chapter 6

6.1. *sAPPα Regulates the Dynamic Control of AMPA Receptors at the Cell Surface*

Previous work from our laboratory, using acutely prepared hippocampal slices, has shown that sAPPα (1 nM, 30 min) increases cell surface expression of GluA1- but not GluA2- containing AMPAR in a manner dependent on protein synthesis (Mockett et al., 2019). Additionally, work from primary hippocampal cultures has provided evidence that sAPPα (1 nM, 120 min) enhances cell surface GluA1 in a protein synthesis-dependent manner (Elder, et al., 2017). Further, in Chapter 5 (refer section 5.1.3. *CP-AMPAR Contribute to the Initial Enhancement of sAPPα-LTP*) we have provided evidence that these GluA1-containing AMPAR are Ca^{2+}-permeable and facilitate the induction of LTP. This chapter aims to expand these observations with specific examination of somatic and dendritic cell surface AMPAR populations. In addition to this, we have extended the treatment window in order to observe the occurrence and persistence of cell surface AMPAR populations across time. In order to investigate both total and *de novo* populations of cell surface AMPAR, we employed the newly developed techniques FUNCAT-PLA and BioPLAy (Elder, 2017) with fluorescence microscopy.

6.1.1. *sAPPα Promotes the Rapid and Persistent Expression of Cell Surface GluA1 in the Soma and Dendrites of Primary Hippocampal Neurons*

We first aimed to examine total populations of cell surface GluA1-containing AMPAR using BioPLAy (refer section 2.3.5. *Detection of Cell Surface Proteins Using BioPLAy*). This technique allows for isolation of proteins present at the cell surface via biotinylation and subsequent PLA. Using BioPLAy, cell surface GluA1 was found to increase in the soma following 30-minute sAPPα treatment (4.41 ± 4.73; $p \leq 0.0001$) compared to control, followed by a small increase 2 hours later (1.65 ± 1.44; $p = 0.0199$; Figure 6-1A). Conversely, dendritic cell surface GluA1 was found to significantly increase within 30 minutes (2.60 ± 1.84; $p \leq 0.0001$), and increase to a greater extent following 2-hour sAPPα treatment (6.64 ± 8.99; $p \leq 0.0001$; Figure 6-1B). Together, these data suggest that sAPPα promotes rapid cell surface expression of GluA1 subunits at the soma and dendrites. These AMPAR, with time, may be trafficked to dendrites via surface diffusion. Alternatively, rapid somatic cell surface expression may be followed by internalisation, transport to dendrites and exocytosis at the dendritic cell surface. While these experiments do not address whether these subunits are newly made or derived from activation of internal pools, these observations extend the results from acute tissue, with an additional level of temporal and spatial resolution.

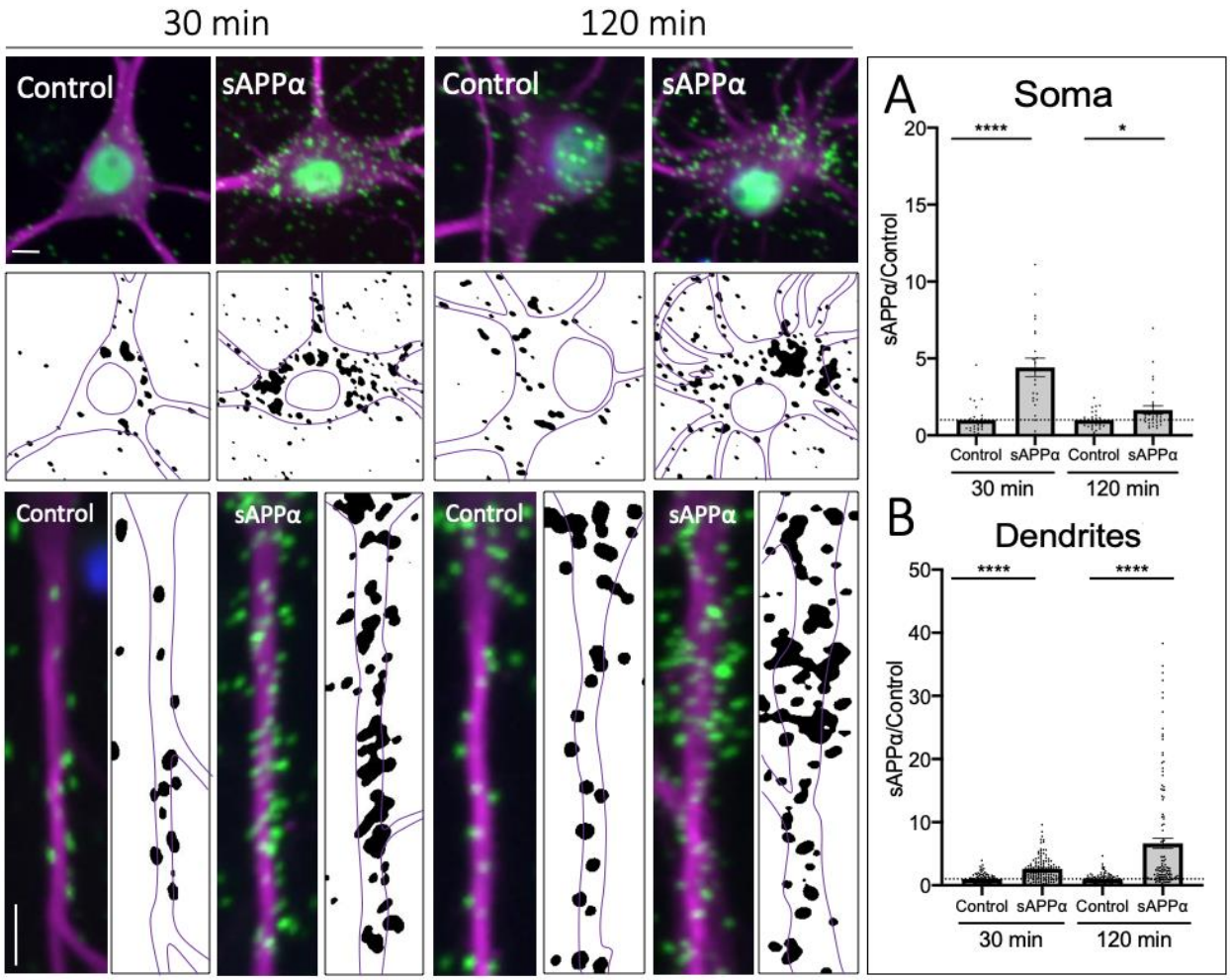


Figure 6-1 | sAPPα enhances cell surface expression of GluA1. Representative images showing cell surface GluA1 levels in the soma (top panels) and dendrites (lower panels) from 30 minute (left) and 120 minute (right) controls and sAPPα treated conditions. A) Average data showing 1 nM sAPPα (30, 120 min) promotes an increase in the soma (n = 30 cells), B) Average data showing 1 nM sAPPα (30, 120 min) promotes an increase in the dendrites (n = 104-128 dendrites). Outliers were removed from each experiment prior to amalgamation using Grubb's tests, and normality was detected by D'Agostino and Pearson omnibus normality tests. All data are expressed relative to control, as mean ± SEM from 3 experiments. Significance was assessed by student's t-test, $^{*}p = 0.0199$, $^{****}p \leq 0.0001$. Representative images show neuronal soma (upper panels) and dendrites (lower panels); MAP2; ■, GluA1; ■, DAPI; ■) Scale bars = 10 µm.

6.1.2. *sAPPα Promotes the Rapid and Transient Trafficking of de novo GluA1-Containing AMPAR to the Somatic and Dendritic Cell Surface*

Previous work in acute hippocampal slices has shown that the rapid increase in cell surface expression of GluA1, following sAPPα-treatment is dependent on protein synthesis (Mockett et al., 2019). This finding suggests the dependence on protein synthesis may arise from either the need to rapidly synthesize GluA1 subunits for subsequent trafficking to the cell surface, or the synthesis of chaperone or scaffolding proteins to aid the trafficking and anchoring of pre-existing, GluA1-containing AMPAR at the cell surface. Alternatively, the protein synthesis component of this may require aspects of both. Therefore, we hypothesised that sAPPα may enhance the rapid synthesis and cell surface expression of GluA1-containing AMPAR in primary hippocampal cultures. To address this, we utilized FUNCAT-PLA under detergent-free conditions.

We found that 30 minutes of sAPPα treatment (1 nM) significantly increased cell surface *de novo* GluA1 in the soma (2.018 ± 2.60; $p = 0.0039$; Figure 6-2A) and dendrites (3.97 ± 10.62; $p = 0.0009$; Figure 6-2B) of cultured hippocampal neurons. However, following 2 hours of sAPPα treatment, there was no detectable change in *de novo* somatic (0.83 ± 1.11; $p = 0.265$; Figure 6-2A) or dendritic (0.86 ± 3.51; $p = 0.673$; Figure 6-2B) GluA1. Indeed, GluA1 was significantly decreased relative to 30 minutes of sAPPα treatment in both the soma ($p = 0.0058$) and dendrites ($p = 0.0005$). These results suggest that sAPPα rapidly induces *de novo* synthesis of GluA1 subunits, which are trafficked to the cell surface by 30 minutes, but are later internalized within 2 hours of treatment.

Of note, is the large standard deviation especially apparent within the dendrites. As a vast majority of the data points for both control and sAPPα-treated groups were zero, the data were not normally distributed (as assessed by D'Agostino & Pearson normality test). However, as all groups expressed a median value of zero, a non-parametric test was deemed an inaccurate representation of the data. Thus, the use of parametric student's t-test is considered appropriate when the sample size is large (Girard et al., 2007; Dieterich et al., 2010; Boos and Stefanski, 2013; Zhang et al., 2015; tom Dieck, et al., 2015). Additionally, calculated variance of our data appears extremely high, however the similar means of experimental groups supports the similar spread of data generated following statistical analysis (CV: control (30 min): 401.3%, sAPPα (30 min): 267.2%, control (120 min): 302.6%, sAPPα (120 min): 406.8%). The variance in our data may be explained by the heterogeneous nature of neuronal cell types present in the primary cell cultures. While we focused on MAP2-positive neurons, this level of analysis does not distinguish between excitatory or inhibitory neurons or indeed pyramidal cells vs. granule cells. Thus, our data may reflect the intrinsic cell-to-cell variability in factors including gene expression, synapse number, receptor abundance, and excitability (Nusser et al., 1998; McAllister et al., 2000; Cullen et al., 2010; Biffi et al., 2013; Pelkmans et al. 2013; Zoli et al., 2018; Osorio et al., 2019).

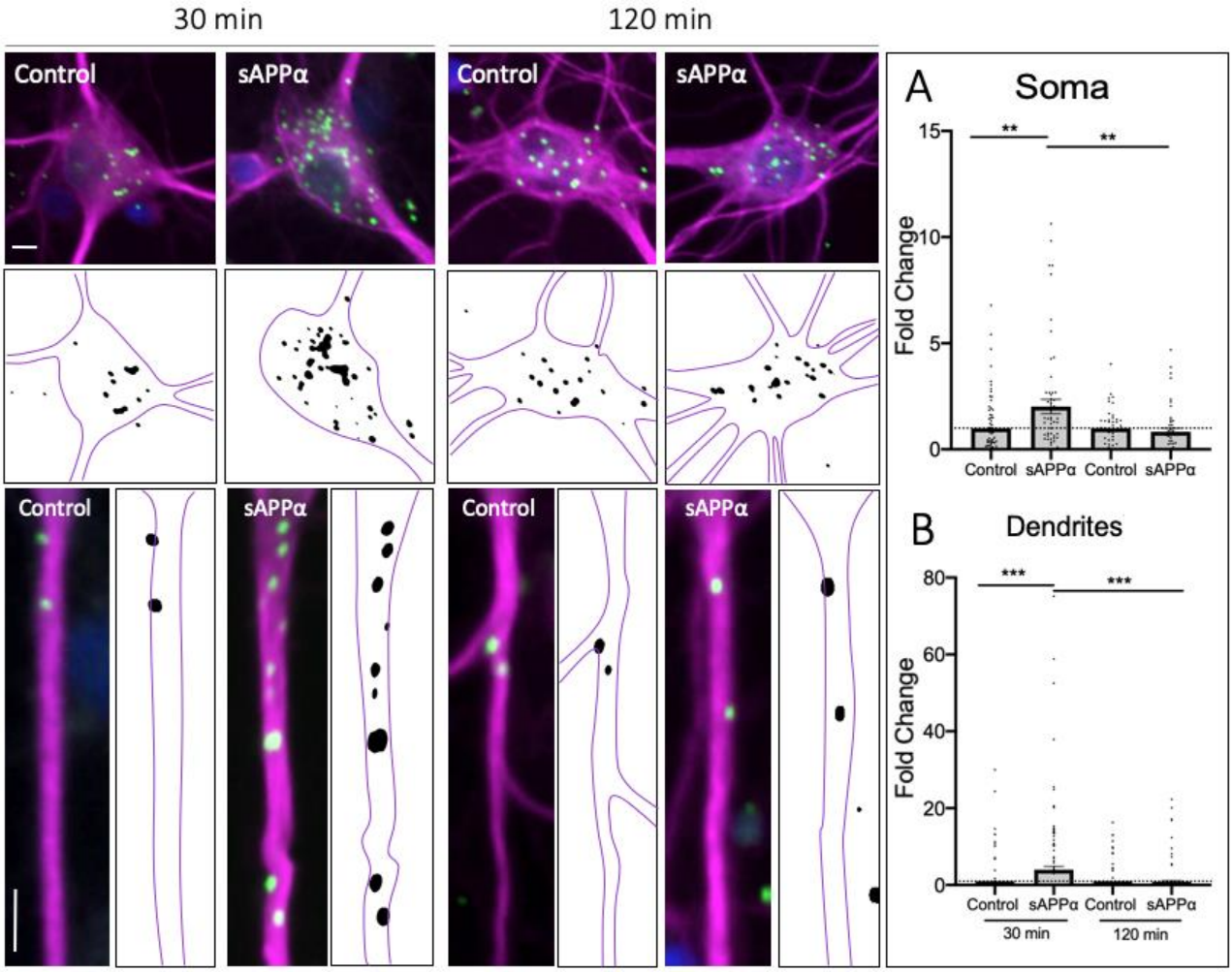


Figure 6-2 | Rapid increase in cell surface *de novo* GluA1 following treatment. Representative images showing cell surface *de novo* GluA1 levels in the soma (top panels) and dendrites (lower panels) from 30 minute (left) and 120 minute (right) control and sAPPα-treated conditions. A) Average data showing 1 nM sAPPα promotes an increase in the soma (n = 50-71 cells) following 30- but not 120-min. B) Average data showing 1 nM sAPPα (30 min) promotes an increase in *de novo* GluA1 at the dendritic cell surface follow 30- but not 120- minute treatments (n = 104-128 dendrites). Outliers were removed from each experiment prior to amalgamation using Grubb's tests, and normality was detected by D'Agostino and Pearson omnibus normality tests. All data are expressed relative to control, as mean ± SEM from 3 experiments. Significance was assessed by student's t-test. $^{**}p \leq 0.01$, $^{***}p \leq 0.001$. Representative images show neuronal soma (upper panels) and dendrites (lower panels; MAP2; ■, GluA1; ■, DAPI; ■) Scale bars = 10 µm.

6.1.2.1. *sAPPα Enhances the Extrasynaptic, but not the Synaptic Population of de novo Cell Surface GluA1*

Following the observations that sAPPα (1 nM, 30 min) significantly increases *de novo* cell surface GluA1, we further sought to determine the synaptic localization of these AMPAR. Here, we have used the presynaptic marker synapsin-1 to determine the proportion of synaptic, extrasynaptic, and non-synaptic *de novo* GluA1-containing AMPAR identified by FUNCAT-PLA at the cell surface (Figure 6-3A, B).

Synaptic overlap of GluA1 puncta was determined using Mander's overlap coefficient (MOC, Figure 6-3C). Here, we found no significant difference between control and sAPPα-treated (1 nM, 30 min) conditions (control: 0.35 ± 0.29, sAPPα: 0.31 ± 0.23; $p = 0.7038$; Figure 6-3C), indicating that sAPPα treatment does not increase the proportion of synaptic GluA1, reflecting the observed lack of effect of sAPPα on basal synaptic transmission in acute hippocampal slices (refer section 5.1.1. *CP-AMPAR Contribute a Small Fraction of Basal Synaptic Transmission*). Due to this, we next sought to determine the proportion of synaptic, extrasynaptic and non-synaptic cell surface *de novo* GluA1 puncta relative to the proximity of synapsin-1-positive synapses. Here, we have defined synaptic, extrasynaptic, and non-synaptic PLA as 0-2, 2-4, and > 4 µm from the closest synapsin-1 centre of mass, respectively (refer section 2.6.3. *Image analysis* and Appendix 1.4. *Quantifying Colocalization of PLA Signal Within Synapses*). Here, we observed a shift in the frequency of *de novo* GluA1 present at synapses following sAPPα treatment, increasing the proportion of GluA1 puncta 2-4 µm proximal to the synapse (Figure 6-3D). Expanding on this, we found a significant increase in the number of *de novo* GluA1 at extrasynaptic sites (control: 1.22 ± 0.089; sAPPα: 1.92 ± 0.14; $p = 0.0003$), however no change was detected at synaptic (control: 1.28 ± 0.10, sAPPα: 1.34 ± 0.12; $p = 0.9872$), or non-synaptic (control: 1.52 ± 0.094, sAPPα: 1.44 ± 0.10; $p = 0.9655$) sites (Figure 6-3E). These results suggest that sAPPα rapidly enhances the extrasynaptic pool of *de novo* GluA1-containing AMPAR within the dendrites.

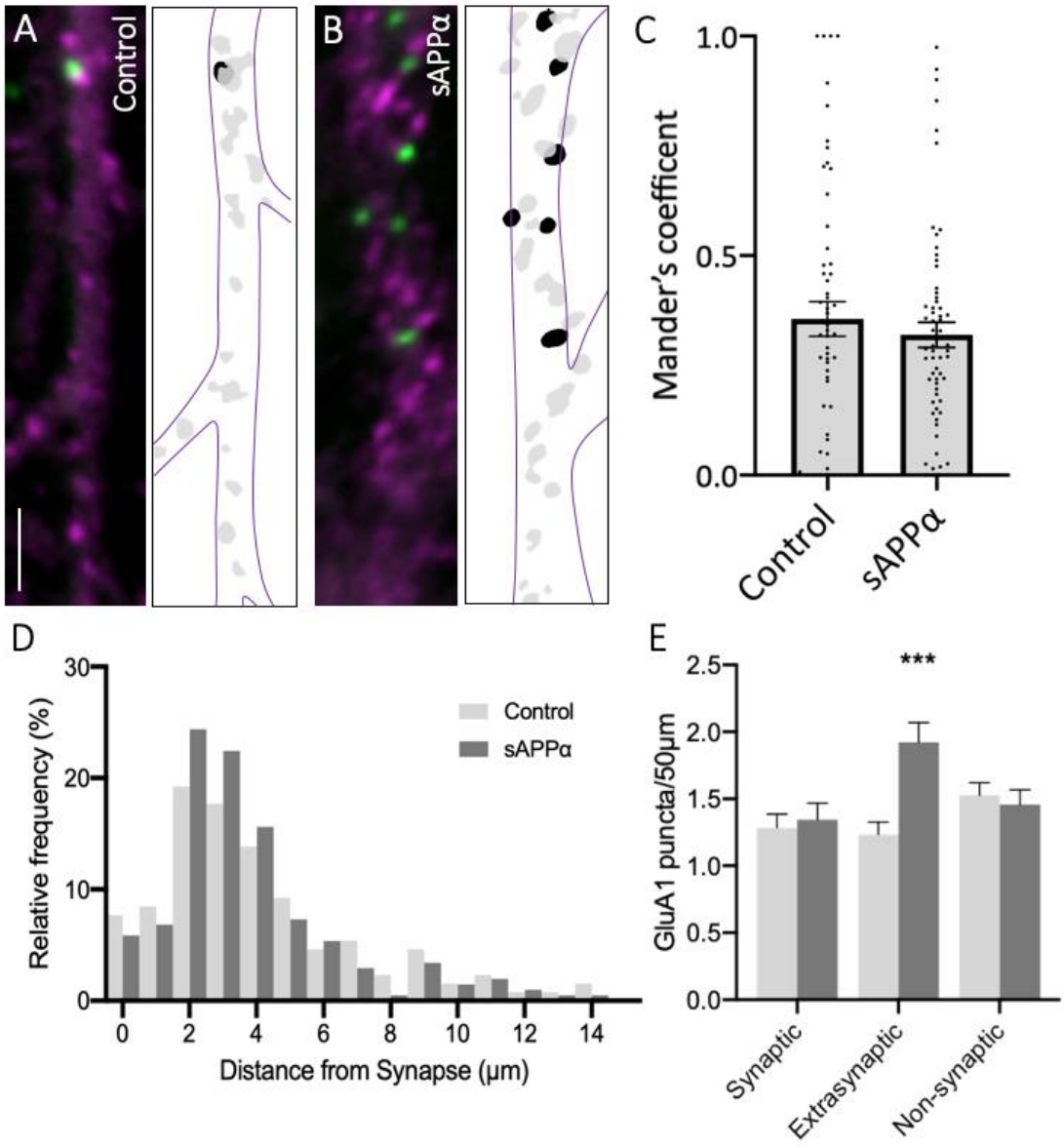


Figure 6-3 | sAPPα enhances *de novo* GluA1 at the extrasynaptic membrane. Representative images showing *de novo* cell surface GluA1 in A) control and B) sAPPα-treated conditions Representative images show dendrites (50 µm; synapsin-1; ■, GluA1; ■). Scale bar = 10 µm. C) No significant difference was observed In the Mander's overlap coefficient following sAPPα-treatment (1 nM, 30 min; *n* = 20 cells, 58-65 dendrites). Significance was assessed by Mann-Whitney two-tailed u-test. D) Frequency histogram of the distribution of *de novo* GluA1 puncta in relevance to synapsin-1 immunofluorescence. Distances were calculated for individual PLA puncta with respect to the closest synapsin-1 centre of mass. Synaptic PLA showed overlapping centres within 0-2 µm, puncta within 2-4 µm were considered extrasynaptic, and puncta beyond 4 µm were considered non-synaptic (*n* = 58-65 dendrites, 130-205 puncta). E) Quantification of GluA1 puncta abundance at the synaptic, extrasynaptic, and non-synaptic membrane (*n* = 58-65 dendrites, 130-205 puncta). Normality was determined by Shapiro-Wilk normality test. Significance was assessed by two-way ANOVA and Šidák's multiple comparisons, $^{***}p \leq 0.0005$.

6.1.3. *Rapid Decrease in Dendritic de novo Cell Surface GluA2 Following sAPPα Treatment*

So far, we have shown that sAPPα (1 nM, 30 min) enhances the cell surface population of *de novo* GluA1. Previously we have shown that expression of total *de novo* GluA2 decreases within the dendrites of neurons following sAPPα treatment, in primary hippocampal neurons (1 nM, 120 min; Elder, 2019), while cell surface GluA2 is unaffected by treatment in acute hippocampal slices (30 min; Mockett et al., 2019). Together, these observations indicate that sAPPα may enhance the cell surface expression of *de novo* GluA1 homomeric receptors, in a manner which may restrict the synthesis or trafficking of GluA2. To further these findings, we sought to examine the expression of *de novo* GluA2 on the cell surface across time, in both somatic and dendritic compartments. Here, we provide evidence that *de novo* cell surface GluA2 remains unaffected by sAPPα-treatment at the soma, following both 30-minute (0.86 ± 1.08, p = 0.654) and 120-minute (1.32 ± 1.22, p = 0.148; Figure 6-4A) treatments. Interestingly, 30-minute treatments showed an early decrease of *de novo* cell surface GluA2 at the dendritic cell surface (0.54 ± 1.45, p = 0.009), returning to control levels within 120 minutes (1.92 ± 9.13, p = 0.395; Figure 6-4B). From this, we can infer that sAPPα may inhibit or otherwise restrict the early expression of *de novo* GluA2 from the cell surface while specifically upregulating the expression of *de novo* GluA1. Previous research has shown that the restriction of GluA2 trafficking by auxiliary proteins such as PICK1 permits the expression of GluA1-containing CP-AMPAR (Hanley et al., 2002; Hanley, 2007; Makuch et al., 2011). Further, we have previously shown that sAPPα (1 nM, 15 min) promotes the expression of the microRNA (miRNA) mir-30 (Ryan et al., 2013), which has been linked to the downregulation of GluA2 in the hippocampus (Song et al., 2019). Together, these results indicate that sAPPα may act to upregulate extrasynaptic *de novo* GluA1-containing homomeric AMPAR in a manner which does not require the association with *de novo* GluA2, further supporting our hypothesis that sAPPα enhances the expression of homomeric GluA1-containing CP-AMPAR.

Similar to data described in section 6.1.2. *sAPPα Promotes the Rapid and Transient Trafficking of de novo GluA1-Containing AMPAR to the Somatic and Dendritic Cell Surface,* a vast majority of the data points within the dendrites for both control and sAPPα-treated groups were zero. Due to the large sample size, a parametric student's t-test is considered appropriate (Girard et al., 2007; Boos and Stefanski, 2013; Zhang et al., 205; tom Dieck, et al., 2015). Calculated variance of our data showed a similar variance across treatment groups (control (30 min): 100.8%, sAPPα (30 min): 178.3%, control (2 hr): 108.0%, sAPPα (2 hr): 92.1%), indicating that while the data were not normally distributed, data were similarly variable across treatment conditions.

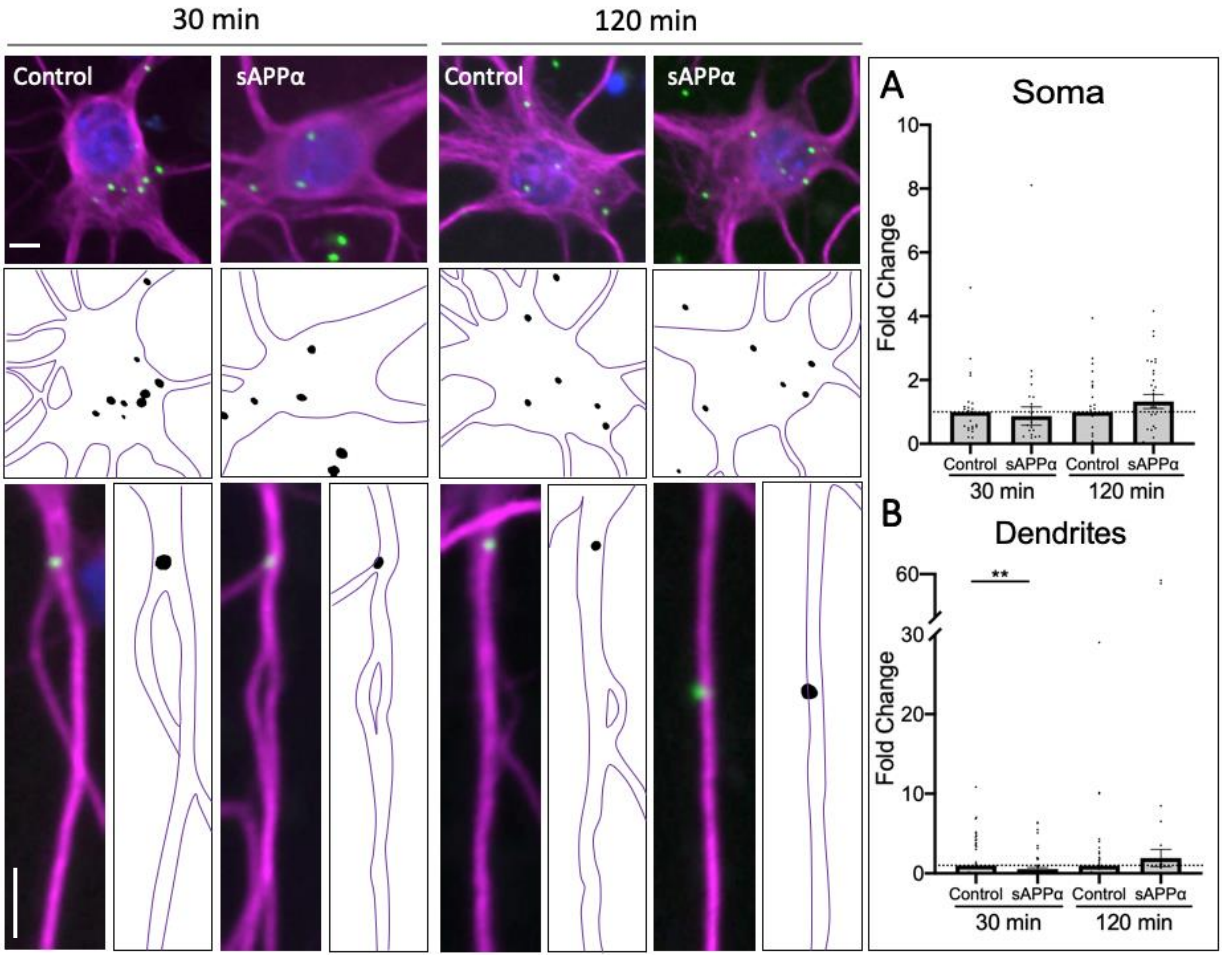


Figure 6-4 | Rapid decrease in *de novo* cell surface GluA2 following treatment. Representative images showing cell surface *de novo* GluA2 levels in the soma (top panels) and dendrites (lower panels) from 30 minute (left) and 120 minute (right) controls and sAPPα treated conditions. A) Average data showing 1 nM sAPPα does not affect somatic (*n* = 25-31 cells) *de novo* GluA2. B) Average data showing 1 nM sAPPα (30 min) significantly decreases dendritic *de novo* GluA2 (*n* = 71-76 dendrites). All data are expressed relative to control, as mean ± SEM from 3 experiments. Outliers were removed from each experiment prior to amalgamation using Grubb's tests, and normality was detected by D'Agostino and Pearson omnibus normality tests. Significance was assessed by student's t-test, $^{**}p \leq 0.01$. Representative images show neuronal soma (upper panels) and dendrites (lower panels; MAP2; ■, GluA1; ■, DAPI; ■) Scale bars = 10 µm.

6.1.4. *Accell™ Arc siRNA Inhibits Somatic and Dendritic sAPPα-Dependent, but not Basal, Arc Expression*

As described above, we have shown that *de novo* GluA1 increases rapidly at the cell surface within 30 minutes of sAPPα treatment, however returning to baseline levels within 2 hours. These results infer a mechanism through which sAPPα promotes the delayed removal of *de novo* GluA1-containing AMPAR previously delivered to the cell surface. Further, we have previously shown that that sAPPα (1 nM, 120 min) enhances Arc protein expression in primary hippocampal neurons (refer section 4.1.2. *sAPPα Facilitates an Increase in Arc Protein Expression;* Livingstone et al., 2019). Due to Arc's close relationship to the internalization of GluA1-containing AMPAR (Shepherd et al., 2006), we hypothesised that Arc may be responsible for the removal of *de novo* GluA1 from the cell surface at a later time-point. In order to examine the expression of *de novo* GluA1 following treatment with the Accell™ Arc antisense siRNA, we first aimed to assess Arc protein expression using immunocytochemistry following treatment of cultures with the Arc siRNA during both basal and sAPPα-stimulated conditions (refer section 2.3.10. *Treatment of Cultures With siRNA* and Appendix 3. *Accell™ siRNA Specificity*). Additionally, we have used the control non-targeting (NT) siRNA with no homology to any known rat gene to assess specificity of the Arc siRNA. In order to ensure effective uptake of siRNA and inhibition of ongoing synthesis, cultures received pre-treatment of siRNA in addition to co-treatment alongside sAPPα.

Following pre-treatment with siRNA (1 µM, 60 min), primary hippocampal cultures were co-treated with either Arc or NT siRNA (1 µM, 120 min), in the presence or absence of sAPPα (1 nM). Treatment of Arc or NT siRNA alone had no significant effect on Arc protein expression in either the soma (Arc siRNA: 0.78 ± 0.53, $p \geq 0.99$; NT siRNA: 1.11 ± 0.48, $p = 0.0509$; Figure 6-5A) nor dendrites (Arc siRNA: 1.26 ± 0.67, $p = 0.8774$; NT siRNA: 1.28 ± 0.47, $p = 0.7561$; Figure 6-5B) relative to control. As shown in *1.3 sAPPα facilitates an increase in Arc protein expression,* sAPPα treatment (1 nM, 120 min) enhanced both somatic (2.05 ± 1.43, $p = 0.0306$; Figure 6-5A) and dendritic (2.33 ± 1.39, $p = 0.0010$; Figure 6-5B) Arc protein expression. This effect was consistent following co-treatment with sAPPα and the NT siRNA (soma: 1.59 ± 1.003, $p = 0.0198$; dendrites: 1.98 ± 0.81, $p = 0.0022$), and no significant difference was detected between sAPPα-treated and sAPPα + NT siRNA in either the soma (sAPPα: 2.05 ± 1.43, sAPPα + NT siRNA: 1.59 ± 1.003, $p \geq 0.9999$ or dendrites (sAPPα: 2.33 ± 1.39, sAPPα + NT siRNA: 1.98 ± 0.81, $p \geq 0.9999$). However, co-treatment of sAPPα with the Arc siRNA significantly reduced Arc protein expression in both the soma (0.72 ± 0.27, $p = 0.0100$) and dendrites (1.16 ± 0.68, $p = 0.0066$) relative to sAPPα-treatment alone, and was not significantly different from control in either compartment ($p \geq 0.99$). Together, these results indicate that the Accell™ siRNA system effectively inhibits *de novo* Arc synthesis in our primary hippocampal cultures.

It is interesting to note that application of the Arc siRNA did not affect basal Arc protein levels. Arc transcription and translation is low under basal conditions (Korb et al. 2011), and therefore small changes from baseline may not be detected by our analyses.

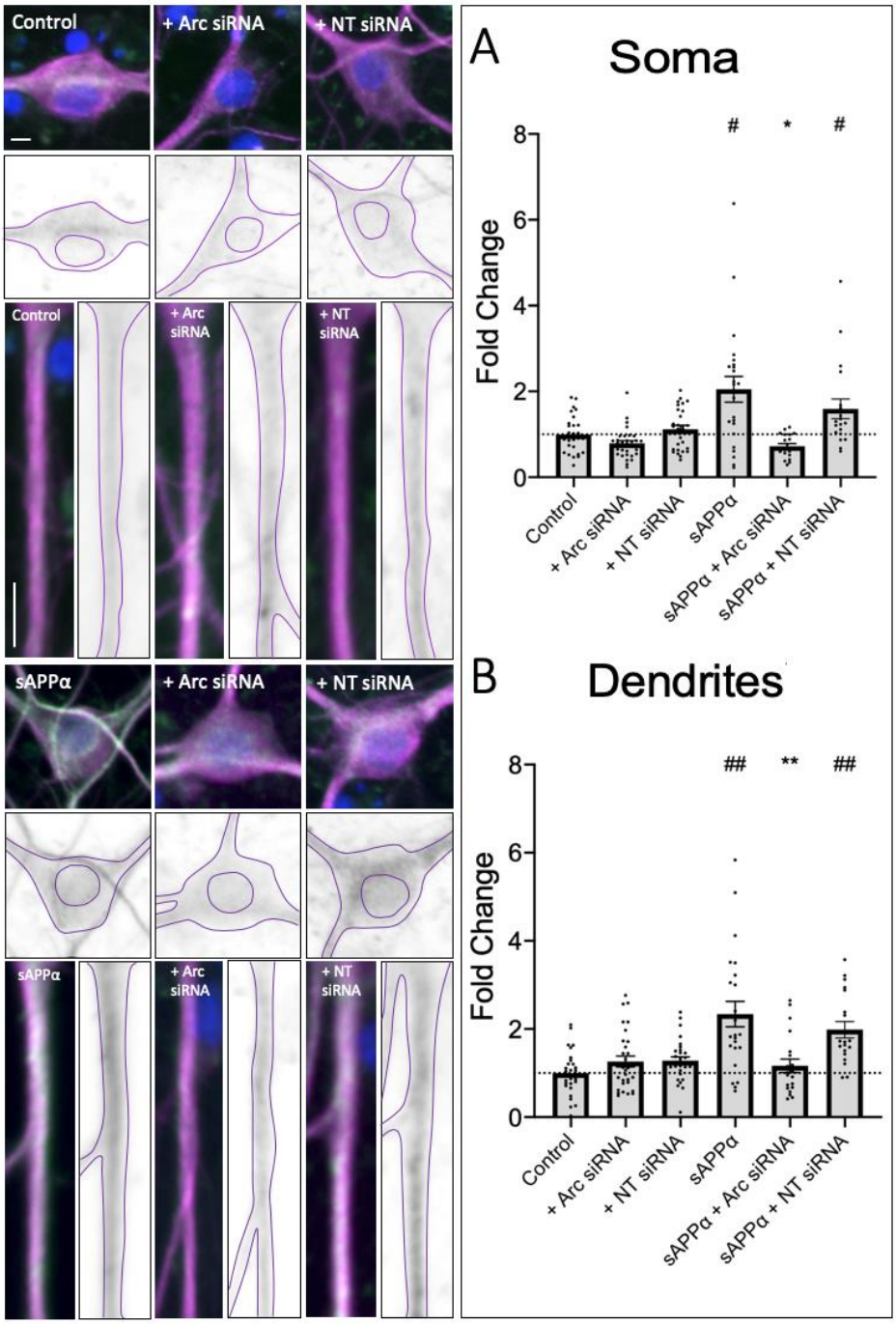


Figure 6-5| sAPPα-promoted Arc expression is affected by Accell™ Arc siRNA. Representative images show Arc protein levels the soma (upper panels) and dendrites (lower panels) in control and sAPPα-treated primary hippocampal neurons ± co-treatment with Arc siRNA or non-targeted (NT) control siRNA. In both the A) soma and B) dendrites (n = 19-30 cells), sAPPα treatment significantly enhanced Arc expression in sAPPα-only and sAPPα + NT siRNA conditions. This effect was inhibited by co-treatment with the Arc siRNA. Outliers were removed from each experiment prior to amalgamation using Grubb's tests, and normality was detected by D'Agostino and Pearson omnibus normality tests. Significance was calculated using a Kruskal–Wallis one-way ANOVA with Dunn's multiple comparisons test on raw data, and expressed as fold change relative to the experimental control from 3 experiments. Hashes (#) denote significant difference from control, asterisks (*) denote significant difference from sAPPα-treated. $^{\#}/^{*}p \leq 0.05$ $^{**}/^{\#\#}p \leq 0.005$. Representative images show neuronal soma, dendrites (MAP2; ■), Arc protein (■), nuclei (DAPI; ■). Scale bars = 10 µm.

6.1.5. *Internalization of de novo GluA1 is Dependent on Arc Expression*

Following the confirmation that treatment of hippocampal cultures with *Accell™* siRNA inhibits sAPPα-stimulated Arc protein expression, we have employed siRNA knockdown of *de novo* Arc protein to assess whether inhibition of Arc protein synthesis results in the persistence of *de novo* cell surface GluA1 expression, as labelled by FUNCAT-PLA, in hippocampal neurons grown in primary hippocampal cultures.

Firstly, as expected, sAPPα treatment for 2 hours resulted in no change in either somatic (0.73 ± 0.88, $p = 0.118$) nor dendritic (0.85 ± 1.56, $p = 0.374$) expression of *de novo* cell surface GluA1, relative to control conditions (see section 6.1.2. *sAPPα Promotes the Rapid and Transient Trafficking of de novo GluA1-Containing AMPAR to the Somatic and Dendritic Cell Surface*). However, in the presence of Arc siRNA (preincubation: 1 µM, 60 min, followed by co-incubation with sAPPα: 1 nM, 120 min), we observed a significant increase in the expression of *de novo* GluA1 at both the somatic (1.55 ± 2.010, $p = 0.0043$; Figure 6-6A) and dendritic (4.14 ± 6.67, $p \leq 0.0001$; Figure 6-6B) cell surface, relative to sAPPα-only conditions. These results suggest that following the rapid expression of *de novo* GluA1 at the cell surface, *de novo* GluA1-containing AMPAR are endocytosed as a result of Arc synthesis.

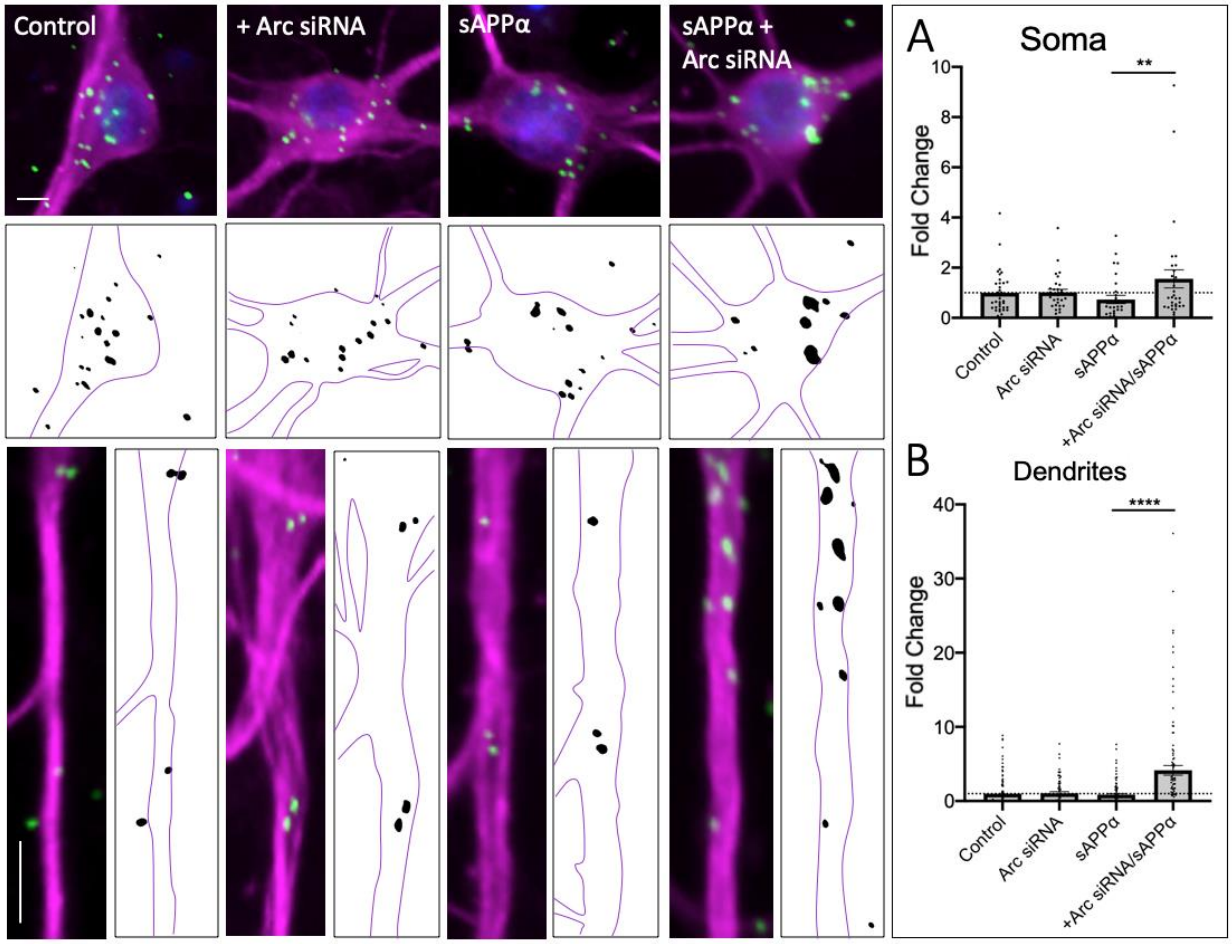


Figure 6-6| sAPPα-induced *de novo* GluA1 persist at the cell surface following siRNA-mediated knockdown of Arc protein. Representative images of somatic (upper panels) and dendritic (lower panels) of control and sAPPα-treated conditions ± treatment with the Arc siRNA. A) Average data showing treatment with sAPPα (1 nM, 120 min) and Arc siRNA (1 µM, pre-treatment: 60 min, co-treatment 120 min) promotes an increase in somatic (n = 28-35 cells) and dendritic (n = 79-132 dendrites) de novo cell surface GluA1. Outliers were removed from each experiment prior to amalgamation using Grubb's tests, and normality was detected by D'Agostino and Pearson omnibus normality tests. All data are expressed relative to control. Data assessing the effect of siRNA are expressed as mean ratio of sAPPα + Arc siRNA/Arc siRNA alone ± SEM from 3 experiments. Significance between control and treatment was assessed by student's t-test, significance between sAPPα-treated and siRNA-treated was assessed by Mann-Whitney two-tailed u-test, $^{**}p \leq 0.001$, $^{****}p \leq 0.0001$. Representative images show neuronal soma (upper panels) and dendrites (lower panels; MAP2; ■, GluA1; ■, DAPI; ■). Scale bars = 10 µm.

6.1.6. *Non-Targeting siRNA has no Significant Effect on de novo Cell Surface GluA1*

In order to assess the specificity of the effects observed following treatment of cultures with Arc siRNA, cultures were similarly treated with the non-targeting (NT) siRNA and processed for FUNCAT-PLA (preincubation: 1 µM, 120 min, followed by coincubation with or without sAPPα: 1 nM, 120 min), in order to examine non-specific effects due to the addition of small nucleotides.

Treatment of cultures with the NT siRNA alone had no observable effect on the levels of *de novo* cell surface GluA1 at the soma (0.94 ± 0.72, $p = 0.7247$; Figure 6-7A), or dendrites (1.083 ± 2.069, $p = 0.7050$; Figure 6-7B). While past experiments have shown a small, non-significant reduction in *de novo* GluA1 at the somatic cell surface following sAPPα treatments (1 nM, 120 min; see section 6.1.2. *sAPPα Promotes the Rapid and Transient Trafficking of de novo GluA1-Containing AMPAR to the Somatic and Dendritic Cell Surface*), here, co-incubation of sAPPα with the NT siRNA showed a small but significant decrease in somatic *de novo* GluA1 soma (0.69 ± 0.50, $p = 0.0224$; Figure 6-7A), however dendritic levels remained unchanged (1.63 ± 2.84, $p = 0.2844$; Figure 6-7B) relative to control conditions. Therefore, the observed effects on cell surface *de novo* GluA1 following Arc siRNA treatment were specific to the addition of Arc siRNA and subsequent knockdown of Arc protein expression, and not as a result of non-specific siRNA-mediated effects.

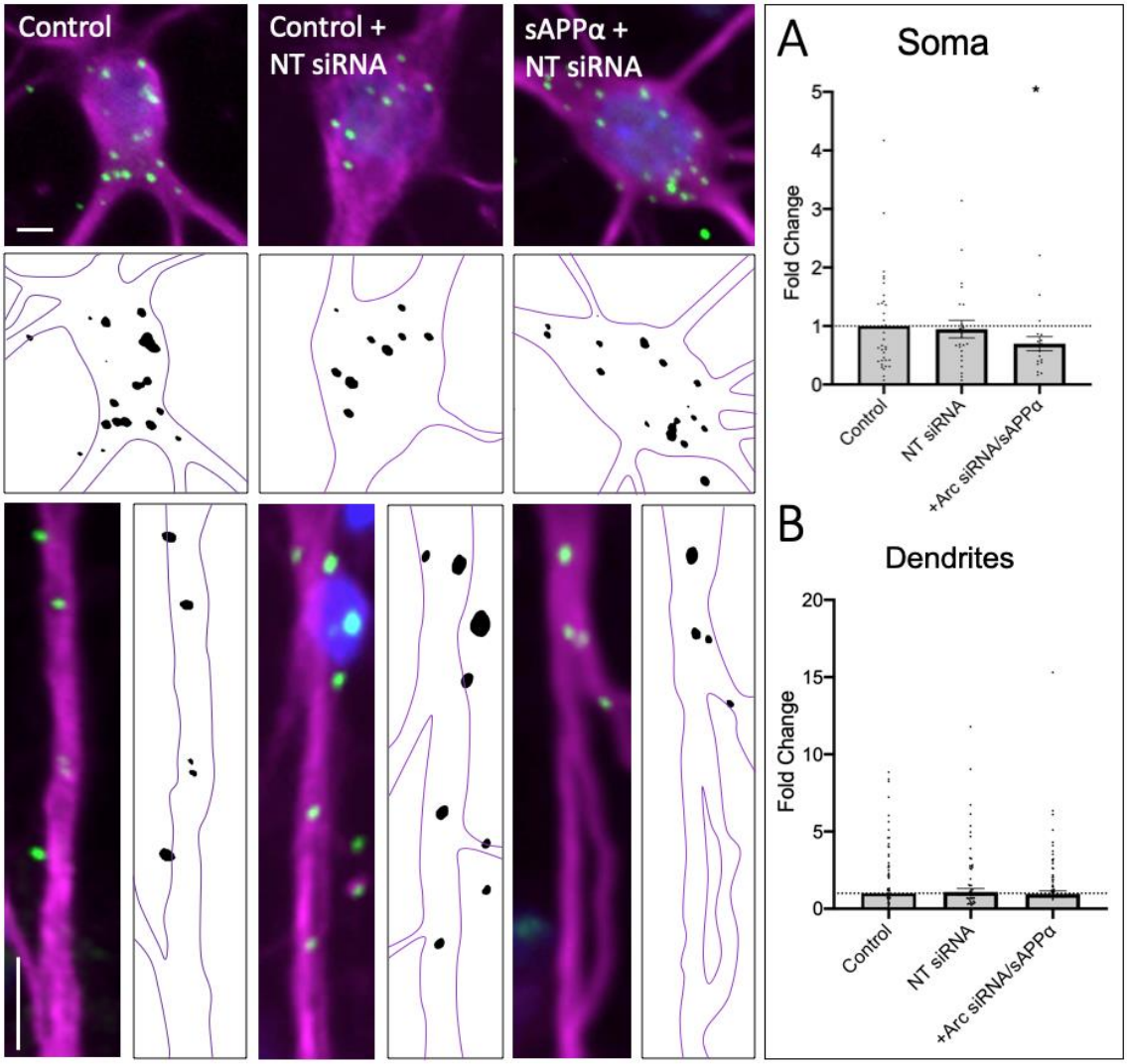


Figure 6-7| Non-targeting siRNA does not affect *de novo* cell surface GluA1. Representative images show cell surface *de novo* GluA1 in the soma (upper panels) and dendrites (lower panels) following treatment with NT siRNA in the presence or absence of sAPPα. Application of NT siRNA alone did not significantly affect expression of cell surface *de novo* GluA1, while NT siRNA in the presence of sAPPα resulted in a small but significant decrease in the A) soma (n = 23-24 cells) but not B) dendrites (n = 89-98 dendrites) cell surface GluA1. All data are expressed relative to the experimental control from 3 experiments. Data assessing the effect of siRNA are expressed as mean ratio of sAPPα + NT siRNA/NT siRNA alone ± SEM. Normality was detected by D'Agostino and Pearson omnibus normality tests and significance was calculated using by student's t-test, $^*p$ = 0.0224. Representative images show neuronal soma, dendrites (MAP2; ■), Arc protein (■), nuclei (DAPI; ■). Scale bars = 10 µm.

6.1.7. *sAPPα Promotes the Delayed Expression of Cell Surface GluA1/2-Containing AMPAR*

While previous research examining acute (30 min) sAPPα treatments (Mockett et al., 2019), as well as the current work described above, has found no evidence for an increase in *de novo* GluA2 synthesis in response to sAPPα, this does not exclude the possibility that sAPPα additionally promotes the trafficking of pre-existing GluA2 subunits to the cell surface. Indeed, in response to synaptic activity (Bagal et al., 2005; Kopec et al., 2006; Guire, et al., 2008; Tanaka and Hirano, 2012; Park et al., 2016; Pandya et al., 2018), behavioural learning (Whitlock et al., 2006; Fachim et al., 2016), and neuromodulators (Leonoudakis et al., 2008; Jourdi and Kabbaj, 2013), cell surface or synaptic accumulation of GluA2-containing AMPAR has been shown to occur. The insertion of these receptors typically follows the incorporation of GluA1-containing homomers. For example, GluA1/2-containing AMPAR have been shown to replace homomeric AMPAR in order to consolidate synaptic potentiation and long-term memories (Shi et al., 2001; Guire et al., 2008; Hong et al., 2013; Park et al., 2016). Therefore, we extended our analysis to examine cell surface populations of GluA1- and GluA2-containing (GluA1/2) AMPAR within somatic and dendritic compartments.

Here we utilized PLA to detect the coincident proximity and expression of GluA1 and GluA2 AMPAR at the cell surface (refer section 2.3.6. *Detection of Cell Surface Receptor Subunit Dimers (PLA)*. Using this technique, we found no significant increase in GluA1/2-containing AMPAR at the cell surface following 30 minute sAPPα treatment, in the soma (0.81 ± 0.58; $p = 0.709$; Figure 6-8A), nor dendrites (1.056 ± 0.92; $p = 0.54$; Figure 6-8B) of cultures primary neurons. However, the cell surface expression of GluA1/2-containing AMPAR significantly increased in both the soma (2.15 ± 1.65; $p = 0.0013$; Figure 6-8A), and dendrites (2.28 ± 1.56; $p \leq 0.0001$; Figure 6-8B) by 120 minutes. These results indicate that GluA1/2-containing AMPAR are expressed at the cell surface following prolonged sAPPα treatment in a manner which adds to, or replaces cell surface GluA1-containing homomers.

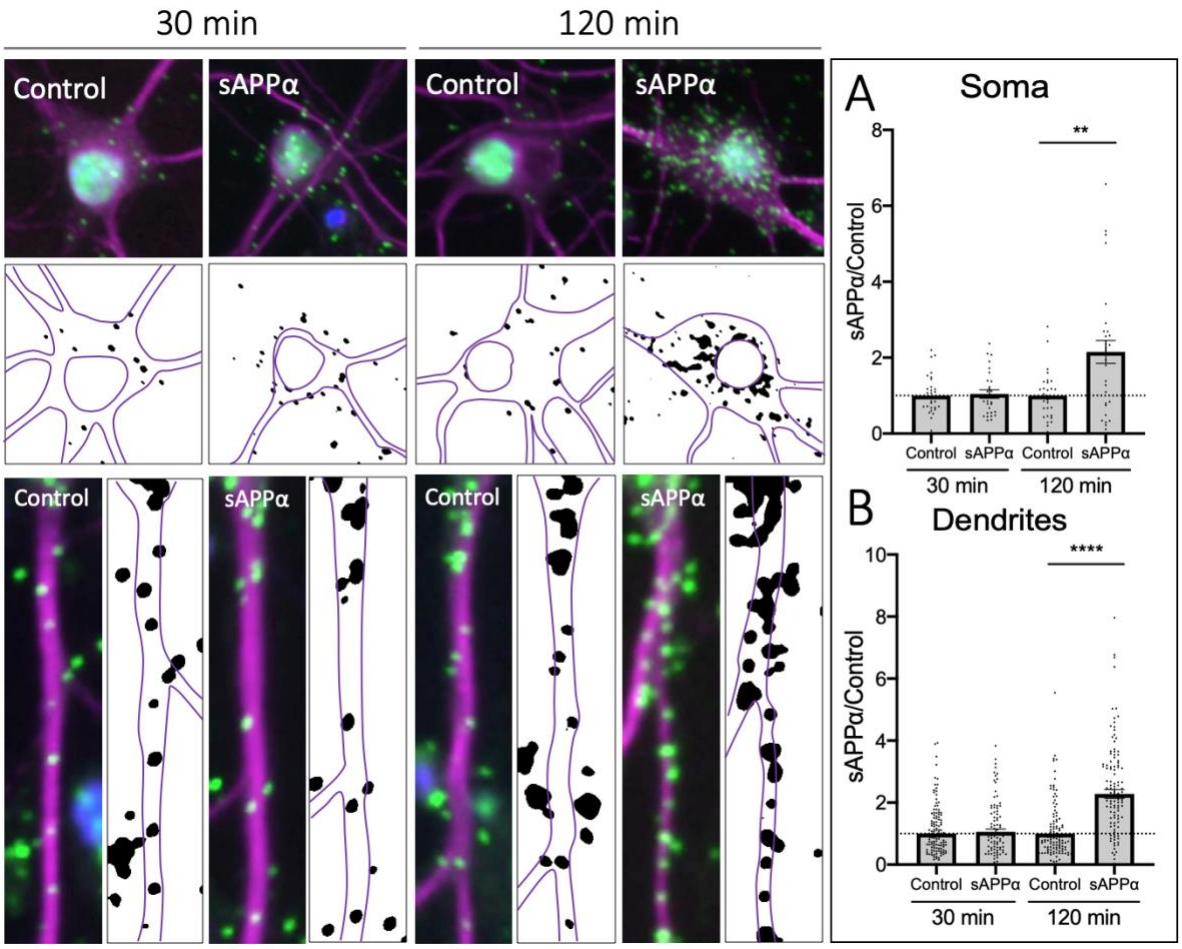


Figure 6-8 | sAPPα enhances cell surface GluA1/2-containing AMPAR following prolonged treatment. Representative images showing cell surface GluA1/2 levels in the soma (top panels) and dendrites (lower panels) from 30 minute (left) and 120 minute (right) controls and sAPPα treated conditions. A) Average data showing 1 nM sAPPα promotes an increase in the A) soma (n = 28-31 cells) and B) dendrites (n = 98-142 dendrites) following 120- but not 30-minute treatment. All data are expressed relative to control, as mean ± SEM from 3 experiments. Normality was detected by D'Agostino and Pearson omnibus normality tests and significance was assessed by student's t-test, $^{**}p \leq 0.001$, $^{****}p \leq 0.0001$. Representative images show neuronal soma (upper panels) and dendrites (lower panels; MAP2; ■, GluA1/2; ■, DAPI; ■). Scale bars = 10 µm.

6.1.8. *sAPPα Promotes the Rapid and Sustained Internalization of GluA2/3-Containing AMPAR*

Following the above findings that sAPPα (1 nM) is able to promote the cell surface expression of heteromeric GluA1/2-containing AMPAR, we sought to further examine cell surface populations of GluA2- and GluA3-containing (GluA2/3) AMPAR; those which comprise the second largest majority of hippocampal AMPAR. The presence of GluA2/3 AMPAR is thought to denote synaptic maturity, with expression increasing throughout development and acting to replace GluA1/2 AMPAR at the synapse via constituent recycling (Zhu et al., 2000; Shinohara and Hirase, 2009). Interestingly, past research has shown that GluA3-containing AMPAR are regulated by *in vivo* synaptic potentiation (Williams et al., 2007), *ex vivo* synaptic depression (Holman et al., 2007), and following *in vitro* growth factor treatment (Narisawa-Saito et al., 1999). Curiously, GluA3-containing AMPAR do not appear to directly regulate the expression of LTP or context fear memory formation (Meng et al., 2003; Humeau et al., 2007), but their removal from the synapse may be an essential step required for LTD (Holman et al., 2007). Regardless, their synaptic expression is considered essential for basal synaptic transmission (Meng et al., 2003).

Using PLA to label cell surface GluA2/3-containing AMPAR, we showed that sAPPα (1 nM) significantly decreases the cell surface expression of GluA2/3-containing AMPAR within the dendrites of cultured neurons (Figure 6-9A,B). Within 30 minutes, sAPPα (1 nM) significantly decreased cell surface GluA2/3 AMPAR expression in the dendrites (0.63 ± 1.28, p = 0.0033; Figure 6-9B), which remained decreased by 2 hours (0.57 ± 1.18, p = 0.0002). Interestingly, somatic levels of GluA2/3 AMPAR remained unaffected following both 30-minute (0.72 ± 1.30, p = 0.255; Figure 6-9A), and 120-minute (1.19 ± 1.81, p = 0.557) treatments. These results may indicate that GluA3-containing AMPAR are removed from the dendritic cell surface to permit the insertion of GluA1- and GluA2-containing AMPAR (Shi et al., 2001; Diering et al., 2017). Alternatively, the removal of GluA3-containing AMPAR may reflect homeostatic processes, maintaining synaptic activity within a physiological range (Rial Verde et al., 2006; Diering et al., 2014; Tan et al., 2015).

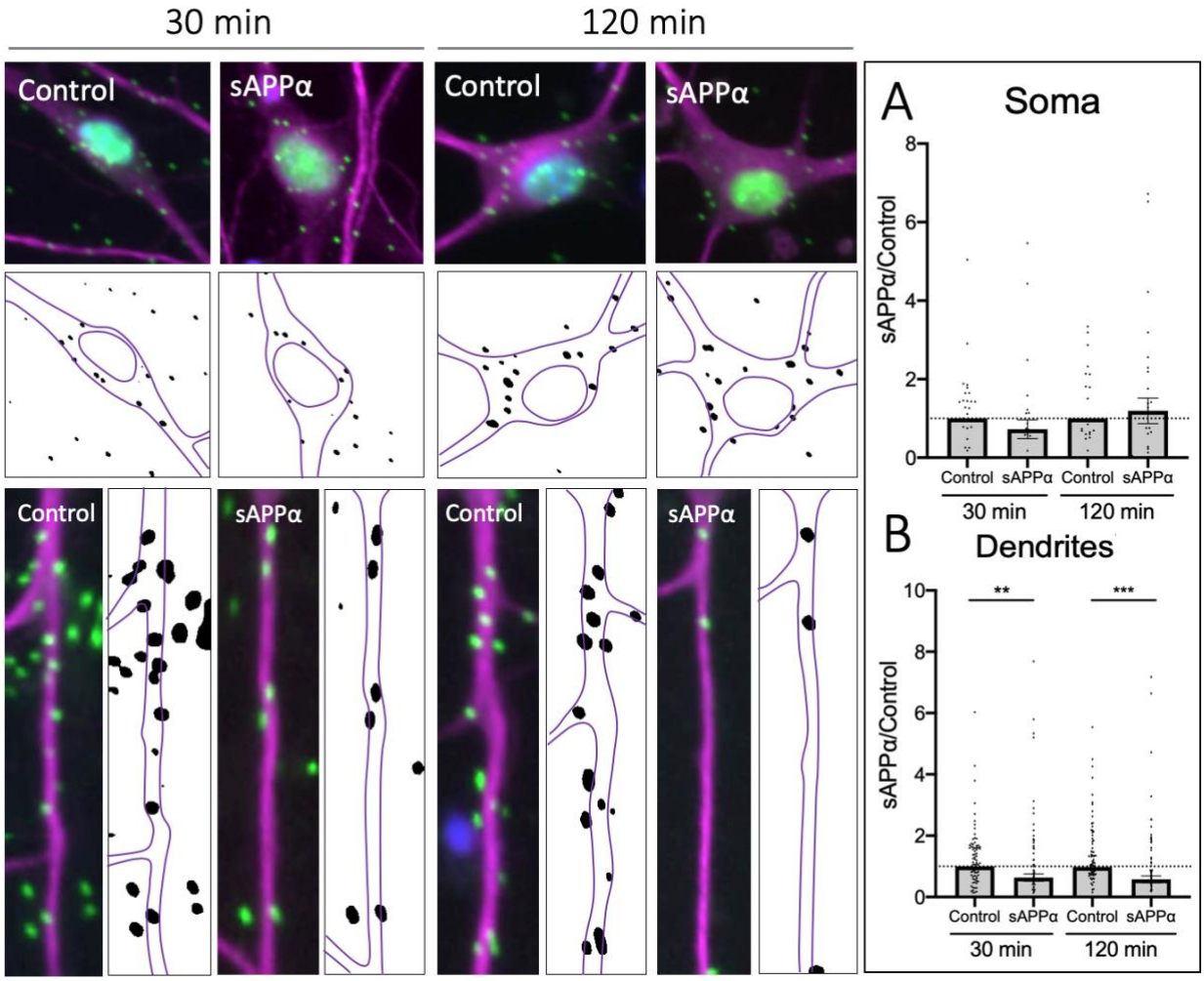


Figure 6-9 | sAPPα decreases cell surface GluA2/3-containing AMPAR expression. Representative images showing cell surface GluA2/3 levels in the soma (top panels) and dendrites (lower panels) from 30 minute (left) and 120 minute (right) controls and sAPPα treated conditions. A) Average data showing sAPPα (1 nM) does not affect somatic GluA2/3 following 30- and 120-minute treatment (n = 30-32 cells). B) Average data showing sAPPα (1 nM; 30, 120 min) promotes a decrease in the dendrites (n = 109-111 dendrites). All data are expressed relative to control, as mean ± SEM from 3 experiments. Normality was detected by D'Agostino and Pearson omnibus normality tests and significance was assessed by student's t-test, $^{**}p$ = 0.0033, $^{***}p$ = 0.0002. Representative images show neuronal soma (upper panels) and dendrites (lower panels; MAP2; ■, GluA1/2; ■, DAPI; ■). Scale bars = 10 µm.

6.2. *Summary*

The work described in this chapter aimed to further examine the trafficking dynamics, and cell surface expression of GluA1-, GluA2-, and GluA3-containing AMPAR. Further, we aimed to examine the specific expression characteristics of *de novo* cell surface GluA1- and GluA2-containing AMPAR, with the hopes of identifying the possible AMPAR composition of newly-synthesised cell surface receptors. Interestingly, we found that sAPPα (1 nM) increased total cell surface GluA1 protein following 30-minute and 2-hour treatments. As well as this, we found a rapid increase in *de novo* GluA1-containing AMPAR within 30 minutes, at extrasynaptic but not synaptic sites. These AMPAR were found to be decreased within 2 hours, possibly indicating a preferential targeting of *de novo* AMPAR over existing AMPAR for endocytosis. In fact, the endocytosis of *de novo* GluA1 was found to be dependent on the synthesis of Arc protein, as incubation of hippocampal cultures with Accell™ siRNA targeting *Arc* mRNA inhibited Arc protein expression, and promoted the persistence of *de novo* GluA1 following prolonged sAPPα treatment. Importantly, while *de novo* GluA1 increased in a time-dependent manner, *de novo* GluA2 did not, supporting the hypothesis that sAPPα promotes the rapid synthesis and trafficking of homomeric GluA1-containing CP-AMPAR to the cell surface. In addition to this we aimed to examine cell surface populations of GluA1/2- and GluA2/3-containing AMPAR. Here we found a delayed increase in GluA1/2 AMPAR at the dendritic cell surface, but a rapid and persistent decrease in GluA2/3 AMPAR. These results may indicate a removal of GluA2/3 AMPAR from the synapse in order to incorporate firstly GluA1-containing CP-AMPAR, and secondly GluA1/2-containing AMPAR.

Chapter 7: Discussion

7.1. *Aims and Objectives*

Dynamic changes in AMPA receptor expression govern changes in neuronal synaptic efficacy, and promote synaptic plasticity. In turn, these changes are thought to underlie information coding and storage in learning and memory processes (Anggono and Huganir, 2012). Of importance, is the regulated synthesis and trafficking of AMPAR to and from the synapse, under strict, activity-regulated guidance (Rumpel et al., 2005). Importantly, many of the mechanisms which govern these processes are negatively affected in neuropsychiatric and neurodegenerative disorders including schizophrenia (Hammond et al., 2010; Corti et al., 2011), major depressive disorder (Maeng et al., 2008; Autry et al., 2011; Sossin et al., 2019), stress and anxiety (Kiselycznyk et al., 2013; Kabir et al., 2017), Parkinson's disease (Chartier-Harlin et al., 2011; Cortese et al., 2016; Zhu et al., 2018), and AD (Langstrom et al., 1989; Ding et al., 2005; Chang et al., 2006; Garcia-Esparcia et al., 2017; Guntupalli et al., 2017; Li et al., 2019). Therefore, much research has been undertaken in order to understand the role of synthesis and trafficking of AMPAR in both health and disease.

This thesis aimed to understand the mechanisms through which sAPPα may regulate LTP and memory, by investigating cell surface AMPAR expression and Arc protein expression within cultured hippocampal neurons, and acute hippocampal tissue. Previously, sAPPα has been shown to enhance LTP (Taylor et al., 2008; Hick et al., 2015), increase cell surface GluA1 (Mockett et al., 2011), upregulate synaptodendritic protein synthesis (Claasen et al., 2009), enhance gene expression (Ryan et al., 2013), and protect against Aβ-related impairments in learning and memory (Seabrook et al., 1999; Xiong et al., 2017; Tan et al., 2018; Morrissey et al., 2019a). Based on these findings, we hypothesised that sAPPα would regulate the enhancement of LTP, in part through the insertion of newly synthesised GluA1-containing CP-AMPAR to the cell surface. Moreover, it was hypothesised that the expression of these AMPAR would be regulated by the expression and function of the IEG Arc. Using a combination of electrophysiology, immunohistochemistry, and FUNCAT-PLA and BioPLAy techniques, we have shown that the enhancement of LTP by sAPPα occurs primarily through the insertion of cell surface and synaptic CP-AMPAR. Further, we have shown that sAPPα enhances the early expression of extrasynaptic *de novo* GluA1- but not GluA2-containing AMPAR at the cell surface, and that their expression is regulated by Arc. Simultaneously, cell surface GluA1/2-containing AMPAR were found to increase at the cell surface within 2 hours, while GluA2/3-containing AMPAR showed a rapid and persistent decrease in response to treatment. Through this, sAPPα appears to utilize the expression and regulation of Arc and AMPAR similar to that of canonical NMDAR- or mGluR-driven LTP, as well as the strengthening of synapses by previously identified growth factors and

neuromodulators, such as BDNF (Caldeira et al., 2007; Kuipers et al., 2016) and dopamine (Sgambato-Faure et al., 2005; Bellone and Lüscher, 2006), however likely through distinct signalling cascades and mechanisms of action. The results discussed within this thesis provide possible explanations and describe critical mechanisms through which sAPPα regulates protein synthesis, Arc expression and AMPAR expression in unique concert to enhance synaptic plasticity within hippocampal neurons.

7.2. *sAPPα Mediates LTP Through the Expression of Ca^{2+}-Permeable AMPA Receptors*

Experience-dependent learning and memory require multiple forms of plasticity at hippocampal synapses (Edelmann et al., 2017). While both NMDAR- (Lüscher and Malenka, 2012) and mGluR-dependent (Bortolotto et al., 1999) LTP comprise much of the our understanding of synaptic plasticity, emerging evidence indicates that GluA1-containing CP-AMPAR may contribute significantly to the expression of certain forms of LTP (Man, 2011).

Here, we sought to examine the contribution of CP-AMPAR to the enhancement of LTP by sAPPα, following the induction of a weak LTP protocol in hippocampal area CA1 of Sprague Dawley rats. Through these experiments, we validated previous research finding that sAPPα enhanced both the induction and persistence of LTP following a mild TBS protocol (Mockett et al., 2019). Importantly, the enhancement of LTP induction following sAPPα treatment was found to be dependent on the synaptic expression of CP-AMPAR (refer section 5.1.3. *CP-AMPAR Contribute to the Initial Enhancement of sAPPα-LTP*). Washout-of IEM-1460 showed a recovery of enhancement indicating that the persistence of potentiation may also be dependent on CP-AMPAR. Interestingly, the induction of LTP following mild TBS alone was found to not comprise a significant CP-AMPAR dependent component (refer section 5.1.2. *CP-AMPAR do not Contribute to LTP Following a Mild Theta-Burst Stimulation Protocol*). These results support the hypothesis that sAPPα mediates the enhancement of LTP induction through the incorporation of CP-AMPAR at the synapse within area CA1 of the adult rat hippocampus.

Although much research has shown an involvement of CP-AMPAR recruitment during LTP (Table 7-1), significant controversy still exists from evidence to the contrary. Importantly, with mounting experimental evidence, it has become apparent that the contribution of CP-AMPAR to LTP is influenced by a number of variables, including most prominently the age (Jensen et al., 2003; Lu et al., 2007b), and species (Gray et al., 2007) of the animals, the recording methods (Plant et al., 2006; Adesnik and Nicoll, 2007), and the induction protocol used (Guire et al., 2008). Previously, CP-AMPAR

dependent LTP has been shown following spaced, but not compressed TBS-induced stimulation (Park et al., 2016) as well as patch-clamp, pre- and post-synaptic pairing protocol (Plant et al., 2006). Notably, both groups found a dependence on CP-AMPAR specific to the induction of LTP, such that application of CP-AMPAR antagonists during spaced tetanisation (Park et al., 2016), or the initial 10 minutes of LTP induction (Plant et al., 2006) impaired both the induction and persistence of LTP. Conversely, application of CP-AMPAR antagonists 20- or 60-minutes post LTP induction did not affect the maintenance of established LTP, indicating that the role of CP-AMPAR in these paradigms transient, yet necessary for LTP induction.

Contrasting this, additional groups have provided evidence that CP-AMPAR are not involved in the induction of LTP. Both Adesnik and Nicoll, (2007) and Gray et al., (2007) have used patch clamp electrophysiology in area CA1 of Sprague Dawley and C57BL/6 mice, respectively, finding an insensitivity to CP-AMPAR antagonists, and no alteration in the rectification index. Importantly, Gray et al., (2007) have shown that the same patch clamp pairing protocol used by Plant et al., (2006) in rats, does not induce a CP-AMPAR dependent LTP in C57BL/6 mice. These results may be explained by anatomical and electrophysiological differences between species, affecting the expression of LTP (Routh et al., 2009; Cao and Harris, 2014). However, these differences are also further influenced by the age of each species. The expression of CP-AMPAR dependent LTP has been linked to mechanisms which enhance the activity of PKA (Park et al., 2016) and CaMKII (Derkach et al., 1999; Terashima et al., 2004). In rodents, PKA dominates early in development (≤ 2 weeks; Yasuda et al., 2003), while a CaMKII-dependent component develops beyond 2 weeks (Wikström et al., 2003). Conversely PKA-dependent LTP may predominate in older, not younger mice (Lu et al., 2007b). Together, these results indicate that the expression of CP-AMPAR is highly regulated, with expression characteristics depending on stimulation type, frequency and pattern, as well as animal model, and thus may reflect the observations from our experiments, such that application of mild TBS alone was not sufficient to induce an IEM-1460-sensitive component of LTP.

Importantly, it is possible that regulation of CP-AMPAR during plasticity events may also require input from other systems (i.e. hormones, neurotrophins, neuromodulators) in some instances. In fact, CP-AMPAR dependent potentiation has been further observed following manipulations *in vivo* including HFS (Williams et al., 2007), brief restraint stress (Whitehead et al., 2017), single-whisker stimulation (Clem and Barth, 2006; Clem et al., 2008; Wen and Barth, 2012), high-fat diet exposure (Suyama et al., 2017), food restriction (Ouyang et al., 2017), irritable bowel syndrome (Liu et al., 2015a), chronic itch (Zhang et al., 2016b), addiction (Conrad et al., 2008), auditory and context fear conditioning (Clem and Huganir, 2010; Hong et al., 2013; Takemoto et al., 2017), as well as some aspects of spatial

memory (Torquatto et al., 2019). In line with this, many molecules that have been investigated have shown diverse roles in regulating AMPAR expression, with the primary aim of enhancing CP-AMPAR expression. These include serotonin (Jitsuki et al., 2011), nicotine (Tang et al., 2015), dopamine (Bellone and Lüscher, 2006; Gao et al., 2006), norepinephrine (Clem and Huganir, 2013), estrogen (Tada et al., 2013; Tada et al., 2015), tumour necrosis factor-α (TNF-α; Leonoudakis et al., 2008), glycine (Jaafari et al., 2012), BDNF (Caldeira et al., 2007; Nakata and Nakamura, 2007; Li and Wolf, 2011; Fortin et al., 2012), as well as drugs such as cocaine (Lee et al., 2013; Ma et al., 2014b; Ma et al., 2016; Wright et al., 2020), methamphetamine (Scheyer et al., 2016) and Δ(9)-tetrahydrocannabinol (THC; Good and Lupica, 2010). The presence of these many regulatory systems governing CP-AMPAR expression may indicate endogenous control of CP-AMPAR by neurotrophic support. Therefore, the application of sAPPα in the current experiments is one of importance. Here, we establish sAPPα as a likewise neurotrophic molecule, capable of modulating synaptic activity through the incorporation of CP-AMPAR.

Following the observation that sAPPα involves a CP-AMPAR dependent component of LTP, an important distinction to be made is the comparison between the mechanism harnessed by sAPPα with those of canonical CP-AMPAR dependent LTP. Past research has shown that the insertion of CP-AMPAR at the cell surface is PKA-dependent (Lu et al., 2007b; Park et al., 2016), while translocation of these AMPAR from the perisynaptic membrane to the synapse is PKC-dependent (Yang et al., 2010). Further, CAMKI has been shown to regulate the trafficking of CP-AMPAR (Guire et al., 2008), while CaMKII governs phosphorylation of GluA1 AMPAR subunits at s831 to enhance Ca^{2+}-permeability or trafficking of CP-AMPAR to the cell surface (Derkach et al., 1999; Terashima et al., 2004).

With relevance to our current observations, previous work has shown that sAPPα enhances LTP through a trafficking-dependent mechanism. The induction of sAPPα-primed LTP is reduced, but not fully eliminated by inhibition of endoplasmic reticulum–Golgi transport, while the maintenance of LTP and expression of cell surface GluA1 is fully eliminated (Mockett et al., 2019). Further, washout of endoplasmic reticulum–Golgi transport inhibitor, BFA, before the application of TBS restored the induction of LTP, indicating that some trafficking was able to occur following sAPPα treatment and before TBS. Additionally, inhibition of endoplasmic reticulum–Golgi transport, *de novo* translation, as well as CaMKII and PKG significantly impair the cell surface expression of GluA1, while inhibition of both CaMKII and endoplasmic reticulum–Golgi transport also significantly reduced the sAPPα-induced increase in s831. Conversely, sAPPα treatment did not affect s845 phosphorylation at the cell surface.

Phosphorylation of s831 has been previously linked to enhancements in single channel conductance (Derkach et al., 1999), likely through the addition of higher conductance CP-AMPAR (Kim

and Ziff, 2014; Yang et al., 2018; Park et al., 2019b; Summers et al., 2019), primarily by CaMKII-mediated signalling. On the other hand, s845 phosphorylation has been associated with increased single-channel open probability as well as promoting GluA1 targeting or retention of AMPAR at the cell surface (Oh et al., 2006; Man et al., 2007; Diering and Huganir, 2018). Interestingly, a PKG-dependent increase in s831 phosphorylation has been found necessary for cocaine-induced increases in locomotor activity (Yang et al., 2018), while cGMP-dependent s831 phosphorylation has been shown to mediate GluA1- but not GluA2 cell surface expression (Cabrera-Pastor et al., 2017). Therefore, these results may imply a mechanism of CaMKII- or PKG-mediated trafficking of s831-phosphorylated CP-AMPAR to the cell surface. Compared to s845, phosphorylation of s831 is enriched in the postsynaptic density (PSD), suggesting that s831 may play a role in targeting to the PSD or stabilization within the PSD (Diering et al., 2017). Together, these results indicate that the priming of LTP by sAPPα is fully dependent on the trafficking of AMPAR to the cell surface and partly dependent on the synthesis of new proteins, while the maintenance of sAPPα-primed LTP is dependent on both mechanisms. Importantly, in both the results observed by (Mockett et al., 2019) and those presented within this thesis, application of sAPPα alone or in conjunction with IEM-1460 did not significantly alter baseline recordings. These observations indicate that sAPPα does not enhance the direct synaptic incorporation of CP-AMPAR but instead increase the extra- or peri-synaptic population of AMPAR to be translocated to the synapse following TBS.

With this in mind, observations from (Park et al., 2019a) have described a similar two-step model for the induction of LTP by CP-AMPAR. Here, it is proposed that initial TBS stimulation activates NMDARs and drives CP-AMPAR from extrasynaptic sites into the synapse to induce the induction of LTP. Simultaneously, NMDAR activation also activates PKA to promote further trafficking of internal CP-AMPAR to the extrasynaptic membrane. Subsequent synaptic activity then drives these AMPAR into the synapse where basal synaptic transmission is able to trigger protein synthesis through the increased influx of Ca^{2+} (Asrar et al., 2009). It has been further proposed that these AMPAR lower the threshold for plasticity at independent, heterosynaptic pathways by promoting the translocation of CP-AMPAR, and local synthesis through PI3K and MAPK activation, ultimately priming synapses for further plasticity.

These results indicate two possibilities; firstly, sAPPα may promote the early synthesis and trafficking of CP-AMPAR to the cell surface. Following TBS stimulation these AMPAR may translocate to the synapse to potentiate the induction of LTP. Alternatively, sAPPα may enhance the trafficking of pre-existing CP-AMPAR from extrasynaptic sites towards the synapse to promote the induction of LTP. Activity at these AMPAR may directly potentiate postsynaptic currents as well as promote activation of downstream Ca^{2+}-sensitive processes including protein synthesis, which may include the trafficking

of *de novo* CP-AMPAR to repopulate extrasynaptic sites. Indeed, it is possible that sAPPα may induce both the trafficking of CP-AMPAR to the cell surface as well as simultaneous movements from perisynaptic sites to synaptic sites to enhance the induction of LTP. Further, while we have shown that sAPPα enhances LTP in a manner dependent on the activation of CP-AMPAR, as discussed above these AMPAR have been shown to regulate a protein synthesis-dependent component of LTP (Park et al., 2018). Therefore, in order to fully understand the role of CP-AMPAR in sAPPα-mediated LTP, future experiments should aim to examine the link between CP-AMPAR and protein synthesis following sAPPα treatment.

Table 7-1 | Summary of literature examining CP-AMPAR in LTP and behavioural paradigms

Author	Animal Species	Age (weeks)	Stimulation protocol						CP-AMPAR Antagonist	CP-AMPAR dependent?
			Trains		Bursts	Interburst (Hz)	Pulse Count	Pulse (Hz)		
Park et al., (2016)	Sprague Dawley	3–12	cTBS	3 (1/10s)	5	20	5	0.01	IEM1460 (30 µm), NSAPM (30 µm), PhTx433 (5–10 µm)	X
			sTBS	3 (1/10min)	5	20	5	0.01		✓
Guire, et al., (2008)	Rats	4-6	HFS	3		5	1	100	IEM1460 (30 µM)	X
			TBS	5		5	4	100		✓
Sanderson et al., (2016)	C57BL6 mice	2	LTD	1	1	100	100	1	IEM1460 (70 µM)	✓
		3								X
Adesnik et al., (2007)	Sprague Dawley	2–3		4	5	100	100	20	PhTx433 (10uM)	X
Lu et al., (2007)	C57BL6 mice	2, 8		2	1		1	100	PhTx-433 (2.5 µM), NASPM (20 µM)	✓
		3-4								✓
		2, 8		1	1		1	100		X
		3-4								X
Asrar et al., (2009)	CD1 GluR2$^{+/+}$	12–24		4	5	100		100	IEM1460 (100uM)	X
	CD1 GluR2$^{-/-}$									✓

Yang et al., (2010)	Sprague Dawley	2-3	2	5	5	5	100	PhTx433 (10 µM), IEM-1460 (50 µM)	✓
			+ postsynaptic depolarisation						
Gray et al., (2007)	C57BL6 mice	8-12	2	1	10	100	100	IEM1460 (100-200uM)	X
		2-3, 3-4,	Pairing Protocol					IEM1460 (100uM)	X
			Membrane potential	Pulses	Hz				
			−10 mV	100	2				
Plant et al., (2006)	Sprague Dawley	3-4	−10–0 mV	50–100	0.5–2 Hz			PhTx 433 (10uM)	✓
Yamanaka et al., (2017)	C57BL6 mice	6–14	+ 30 mV	80	2 Hz			NASPM (50 µM)	✓
Purkey et al., (2018)	C57BL6 mice	2–8	0 mV	2	100			NASPM (20 µM)	X
			0mV	270	3				✓
Cepeda-Prado et al., (2019)	C57BL6 mice	4	Pairing Protocol					NASPM (100 µM)	✓
			Repeats	Action Potential Count	Hz				
			6	1, 4	0.5				
In vivo Behavioural Paradigms									
Clem and Barth, (2006)	C57BL6 mice	1-2	Single-whisker stimulation protocol					Joro spider toxin (10–20 µM)	✓
Clem et al., (2008)	C57BL6 mice	1-2	Single-whisker stimulation protocol					Assessed by rectification	✓
Wen and Barth, (2012)	C57BL6 mice	2	Single-whisker stimulation protocol					NASPM (50 µM), PhTx (10 µM)	✓
Clem and Huganir, (2010)	C57BL6 mice	4-7	Context fear conditioning					NASPM (50 µM)	✓
Whitehead et al.,	Wistar rats	4-5	Brief restraint stress					Spermine (100 mM), IEM: 100 mM	✓

(2017)					
Suyama et al., (2017)	C57BL6 mice	3–5	High-fat diet exposure	IEM 1460 (100 μM)	✓
Ouyang et al., (2017)	Sprague Dawley rats		Food restriction	NASPM (200 μM)	✓
Zhang et al., (2016)	Sprague Dawley rats	3–5	Chronic itch	Assessed by rectification	✓
Conrad et al., (2008)	Sprague Dawley rats	Unknown	Cocaine addiction	NASPM (100-200 μM)	✓
Takemoto et al., (2017)	C57BL6 mice	4	Context fear conditioning	Assessed by rectification	✓
		8			✓
Hong et al., (2013)	Sprague Dawley rats	4-5	Auditory fear condition	NASPM (50 μM)	✓
Torquatto et al., (2019)	Wistar rats	2-3	Auditory fear condition	NASPM (0.4 μM, 4 μM, 40 μM)	✓
			Contextual fear memory	NASPM (0.4 μM)	✓
			Object location task	NASPM (0.4 μM)	X
			Spatial memory test	NASPM (0.4 μM)	✓

7.3. *sAPPα regulates the Trafficking of Existing and Newly-Synthesized AMPAR Receptors*

7.3.1. *GluA1-containing AMPA Receptors*

As discussed previously, past research has shown that sAPPα (1 nM, 30 min) enhances cell surface GluA1 expression in a protein synthesis-dependent manner, in acute hippocampal slices (Mockett et al., 2019). Throughout this thesis, we have further described the requirement of CP-AMPAR at the synapse for the induction of sAPPα-primed LTP (refer section 5.1.3. *CP-AMPAR Contribute to the Initial Enhancement of sAPPα-Mediated LTP*). This evidence suggests either a dependence on the synthesis of GluA1 proteins directly, or synthesis of chaperone or scaffolding proteins to aid the trafficking and anchoring of pre-existing, GluA1-containing AMPAR to the cell surface. Alternatively, the protein synthesis component of this may indeed require aspects of both. To address this, we first sought to determine whether sAPPα (1 nM) enhances cell surface GluA1 in our primary hippocampal neurons.

Using BioPLAy, we found that GluA1 expression at the cell surface was enhanced following sAPPα treatment for both 30 minute and 2-hour incubations (refer section 6.1.1. *sAPPα Promotes the Rapid and Persistent Expression of Cell Surface GluA1 in the Soma and Dendrites of Primary Hippocampal Neurons*). Data from these experiments indicates an early enhancement of GluA1 at the somatic surface, followed by a later increase in the dendrites. While this may indicate distinct mechanisms of somatic and dendritic exocytosis of GluA1-containing AMPAR, it may also result from lateral diffusion from somatic to dendritic compartments (Cognet et al., 2006; Earnshaw and Bressloff, 2008). Of interest, work from Adesnik et al., (2005) has made comparisons between the recycling rates of somatic and dendritic recycling pools, finding that intracellular dendritic AMPAR cycle to the synapse on the scale of hours, while trafficking from internal stores to extrasynaptic somatic sites occurs on the scale of minutes. These newly inserted AMPAR are then capable of lateral travel along dendrites to reside at synapse. Given a coefficient of Brownian diffusion of 0.01–0.5 $\mu m^2/s$ (Tardin et al., 2003), an AMPAR at the cell body could take up to 1.5 hours to travel 50 µm, placing it in the timescale of our experiments. Of consideration is the saturating nature of the mean square displacement, such that with time roughly 25% of freely diffusing extrasynaptic receptors display spatially restricted diffusion within a domain size of ~300 nm, or the equivalent of a PSD (Kusumi et al., 1993; Tardin et al., 2003). Thus, diffusion from the soma follows an exponential fit as laterally diffusing AMPAR track towards and are captured by, or reside within, perisynaptic and synaptic spaces. Regardless of the mechanism of action, we can conclude that *in vitro* treatments of

sAPPα increase the cell surface population of GluA1-containing AMPAR in a manner similar to previous *ex vivo* experiments (Mockett et al., 2019).

Similarly, using FUNCAT-PLA we have also shown that cell surface populations of *de novo* GluA1 are dynamically regulated following sAPPα treatment (refer section 6.1.2. *sAPPα Promotes the Rapid and Transient Trafficking of de novo GluA1-Containing AMPAR to the Somatic and Dendritic cell surface*). Here, we found that short (30 minute) but not long (2 hour) treatments of sAPPα significantly increased newly synthesised GluA1-containing AMPAR at both the somatic and dendritic cell surface. Interestingly, *de novo* GluA1 at 2 hours was significantly reduced in both compartments returning to control levels. These results may be explained through a few different possibilities. While direct exocytosis of *de novo* GluA1 may occur at the somatic cell surface (Yudowski et al., 2007), we cannot rule out 3 possible routes for the expression of *de novo* GluA1 at the dendritic cell surface. Firstly, as previously mentioned, somatic *de novo* GluA1 may diffuse along the plasma membrane. However, given the time constraints of AMPAR present within 50 µm of proximal dendrites within 30 minutes, it is unlikely. Secondly, *de novo* dendritic GluA1 may be translated and packaged into vesicles within the somatic Golgi apparatus and trafficked anterogradely along the dendrites at a previously identified mean speed of 1.5 $\mu m/s^{-1}$ (Hangen et al., 2018). This active transport is ten-fold faster than Brownian diffusion at the cell surface, placing the trafficking of *de novo* GluA1 in the dendrites, and the delivery to the cell surface, within our time constraints. Thirdly, while we have not directly assessed the role of local dendritic synthesis in the formation and trafficking of these AMPAR, previous evidence has shown that sAPPα is able to stimulate synaptodendritic protein synthesis (Claasen et al., 2009), and APP has been shown to directly bind the RNA binding protein cytoplasmic polyadenylation element binding protein (CPEB) at the cytoplasmic surface (Cao et al., 2005; Savtchouk et al., 2016). Activation of CPEB has been shown to be important for the local synthesis of GluA1 and GluA2 AMPAR subunits (Si et al., 2003; Pavlopoulos et al., 2011), and the stabilization and persistence of LTP and long-term memory (Fioriti et al., 2015). Therefore, it may be possible that these AMPAR are synthesised locally and inserted directly.

Interestingly, in the current experiments cell surface *de novo* GluA1-containing AMPAR were found to be expressed primarily at extrasynaptic sites (refer section 6.1.2.1. *sAPPα Enhances the Extrasynaptic, but not Synaptic Population of de novo Cell Surface GluA1*). GluA1-containing AMPAR are essential for the formation of extrasynaptic pools of AMPAR, such that $GluA1^{-/-}$ mice show little to no extrasynaptic AMPAR populations (Andrásfalvy et al., 2003; Lisman and Raghavachari, 2006), and the expression of extrasynaptic AMPAR has been deemed necessary for the induction of LTP (Oh et al., 2006; Penn et al., 2017), while increasing the proportion of extrasynaptic AMPAR primes synapses

for LTP (Oh et al., 2006). These results may further support the role of sAPPα in the priming of LTP, through the expression of *de novo* GluA1-containing AMPAR at extrasynaptic domains.

These results are further corroborated by observations of similar neurotransmitters and neuromodulators, including glutamate, dopamine, BDNF, and the mGluR agonist DHPG, all of which have been shown to stimulate local protein synthesis (Kacharmina et al., 2000; Ju et al., 2004; Smith et al., 2005; Claasen et al., 2009; Leal et al., 2014). Of interest, glutamate, dopamine and BDNF have been found to promote the local synthesis and trafficking of GluA1, but not GluA2-containing AMPAR and their trafficking to the cell surface within 30 minutes (Smith et al., 2005; Sutton et al., 2006; Caldeira et al., 2007; Mameli et al., 2007; Li and Wolf, 2011; Fortin et al., 2012). Interestingly, in response to dopamine, GluA1-containing AMPAR have been shown to traffic directly to the synapse of AMPA-silent synapses in DIV 14-21 cultured hippocampal neurons (Smith et al., 2005), as well as enhance the extrasynaptic population of GluA1-containing AMPAR in DIV 14-21 cortical neurons (Gao et al., 2006). In the latter of these experiments, subsequent NMDAR activation was found to promote the trafficking of these extrasynaptic AMPAR to the synapse. Importantly, application of both BDNF (Akaneya et al., 1997; Ying et al., 2002; Ji et al., 2010) and dopamine (Shetty et al., 2017) to acute hippocampal slices potentiates the EPSP in the absence of tetanic stimulation. Interestingly, subsequent induction of LTP is impaired following BDNF-induced enhancement of baseline synaptic transmission, indicating that these AMPAR are susceptible to removal by depotentiation-like mechanisms, or saturation of AMPAR responses. These observations distinguish both BDNF and dopamine from sAPPα, such that application of sAPPα to acute hippocampal slices has no effect on baseline synaptic transmission (refer section 5.1.1. *CP-AMPAR Contribute a Small Fraction of Basal Synaptic Transmission*; Richter et al., 2018; Mockett et al., 2019), indicating that the trafficking of these AMPAR occurs at primarily extrasynaptic sites, and requires the induction of LTP to promote their synaptic localization. Therefore, while sAPPα appears to employ distinct mechanisms to that of BDNF and dopamine, the induction of protein synthesis and CP-AMPAR trafficking may be a common underlying factor of these neuromodulators

7.3.1.1. *Regulation of de novo GluA2-containing AMPA Receptors*

Following the observation that *de novo* GluA1-containing AMPAR increased at the cell surface and that CP-AMPAR provide a significant contribution to sAPPα-enhanced LTP, we further sought to determine the identity of these AMPAR by likewise examination of *de novo* cell surface GluA2. Here, we found no significant increase in cell surface *de novo* GluA2 following 30 minute or 2-hour incubations with sAPPα, indicating these AMPAR are GluA1 homomers. In fact, we found a small but significant decrease in *de novo* GluA2 at the dendritic cell surface, within 30 minutes of treatment.

These results support earlier observations from our lab that *de novo* GluA2 is decreased in the dendrites following 2-hour treatments (Elder et al., 2019). This may indicate one of two possible mechanisms. Firstly, GluA2 synthesis may be directly inhibited during this time. As previously mentioned, CPEB has been shown to bind APP and regulate the local synthesis of GluA1 and GluA2, however, CPEB3 has been shown to bind and inhibit translation of *GluA2* mRNA (Huang et al., 2006). Additionally, sAPPα (1 nM, 15 min) has been previously shown to upregulate miR-30 (Ryan et al., 2013), a miRNA which has been linked to the downregulation of GluA2 in the hippocampus (Song et al., 2019). Of note, the upregulation of miR-30 is transient, and was not found to be significantly upregulated following 2 hours of sAPPα treatment (Ryan et al., 2013), reflecting the transient decrease in GluA2 in the current experiments. This observation adds an additional possible level of control over the synthesis and formation of AMPAR following sAPPα treatment. Alternatively, active processes may govern the restriction of GluA2 proteins from trafficking to the cell surface. Under basal conditions N-ethylmaleimide-sensitive factor (NSF) inhibits PICK1-mediated intracellular GluA2 retention (Hanley et al., 2002; Hanley, 2007; Makuch et al., 2011), permitting the constitutive cycling of GluA2-containing AMPARs. However, following glycine-induced LTP, increased PICK1-GluA2 interaction within the endosomal compartment inhibits forward trafficking of GluA2, permitting the expression of primarily GluA1-containing CP-AMPAR at the cell surface (Terashima et al., 2004; Bellone and Luscher, 2006; Jaafari et al., 2012). Together, it is possible that either the dendritic translation of GluA2 or the export of *de novo* GluA2 from the soma may be regulated as such to favour export of GluA1-containing AMPAR to the cell surface. Therefore, we can conclude that the rapid synthesis of GluA1-containing AMPAR likely involves the formation of Ca^{2+}-permeable GluA1 homomers.

7.3.2. *GluA1/2-containing AMPA Receptors*

The expression of GluA1/2-containing AMPAR is closely related to the expression and persistence of LTP (Isaac et al., 2007; Díaz-Alonso et al., 2017; Penn et al., 2017; Zhou et al., 2018), and found to be crucial for the maintenance of hippocampal LTP (Penn et al., 2017). Therefore, we have utilized PLA for coincident detection of GluA1 and GluA2 protein at the cell surface of cultured hippocampal neurons, finding a delayed increase in the proportion of GluA1/2-containing AMPAR following 2 hours of sAPPα treatment, at both the somatic and dendritic cell surface. Importantly, GluA2-containing AMPAR have previously been thought to contribute to the switch of GluA1-containing CP-AMPAR to GluA2-containing Ca^{2+}-impermeable (CI)-AMPAR (Shi et al., 2001; Bellone and Lüscher, 2005b; Sutton et al., 2006; Mameli et al., 2007). As previously mentioned, many electrophysiology experiments have described a sensitivity of LTP to CP-AMPAR antagonists during early but not late potentiation (Guire et

al., 2008; Park et al., 2016), likely implicating a switch from CP-dependent to CP-independent potentiation, indicating that early activity at CP-AMPAR is necessary for the expression of later potentiation (Park et al., 2016). This process appears to require interactions between GluA2 and NSF, GRIP, or PICK1, as inhibition of these interactions results in prolonged expression of cell surface CP-AMPAR and LTP governed solely by CP-AMPAR (Clem and Huganir, 2010; Yang et al., 2010).

During memory formation, the incorporation of CP-AMPAR at synapses is thought to place the synapse into a less stable, and more 'labile' state (Hong et al., 2013). Activity at these AMPAR has been found to be required for the reconsolidation of memories, and promote the later exchange for GluA2-containing CI-AMPAR. Interestingly, the initial insertion of synaptic CP-AMPAR may also be dependent on the initial removal of existing CI-AMPAR from the cell surface, as inhibition of CI-AMPAR endocytosis blocked the initial exchange for CP-AMPAR. This may support the idea that synaptic domains contain limited spaces, or 'slots,' for the addition of AMPAR (MacDougall and Fine, 2013).

Interestingly, impairing Arc protein degradation and increasing Arc protein expression in hippocampal cultures results in a dramatic decrease in GluA1-containing AMPAR at the cell surface and a small but significant increase in GluA2-containing AMPAR (Wall et al., 2018), following activation of mGluR. Here, the authors discuss the possibility that mGluR activation promotes the removal and replacement of CP-AMPAR with GluA1/2-containing AMPAR in an Arc-dependent manner. In the current experiments, it is possible that the Arc-dependent removal of *de novo* GluA1-containing AMPAR also permits the trafficking of GluA1/2-containing AMPAR to the cell surface. Such observations warrant further experiments to clarify Arc's role in this mechanism.

7.3.3. *GluA3-containing AMPA Receptors*

GluA2/3-containing AMPAR are the next major heteromer within the hippocampus (Wenthold et al., 1996), and PSD (Pandya et al., 2017). While under certain conditions these AMPAR contribute to LTP (Renner et al., 2017), for the most part GluA3-containing AMPAR exist in low-conductance states, contributing solely to basal transmission (Meng et al., 2003). Due to this, we extended our experiments to cover the quantification of GluA2/3 levels at the cell surface following sAPPα treatment. Unexpectedly, we found an early and prolonged reduction in GluA2/3-containing AMPAR in the dendrites, while somatic GluA2/3 remined unchanged.

As mentioned above, past research has indicated a necessity of GluA2-containing CI-AMPAR internalization before the addition of GluA1-containing CP-AMPAR (Bellone and Lüscher, 2005; Bellone and Luscher, 2006; Hong et al., 2013). Here, it is thought that the number of receptors in a given synapse may be limited by 'slots,' determined by the number of available anchoring proteins of

the PSD (MacDougall and Fine, 2013). Preventing the removal of GluA2-containing AMPAR by inhibiting the GluA2-PICK1 interaction prevents cocaine-induced expression of CP-AMPAR and transition of the synapse into a labile state (Bellone and Luscher, 2006). Importantly, the GluA2-PICK1 interaction is necessary for the endocytosis and lysosomal targeting of GluA2/3-containing AMPAR (Koszegi et al., 2017). Live cell imaging studies reflect these observations, however on shorter time-scales. Total internal reflection fluorescence (TIRF) live-cell microscopy has shown that while GluA1-containing AMPAR increase at the cell surface within 5–15 minutes post LTP induction, GluA2/3-containing AMPAR show a marked decrease within 5 minutes and a gradual recovery upon 15 minutes (Tanaka and Hirano, 2012). While these timescales perhaps do not reflect the results presented here, this may be due to differences in the mechanisms involved between electrical field stimulation and treatment with exogenous neurotrophins, including sAPPα. Similarly, *in vivo* research has shown that HFS stimulation upregulates GluA1 and GluA3 at the cell surface within 20 minutes of stimulation, of which GluA1, but not GluA3 is likewise upregulated in the synapse. Within 20 minutes GluA3 is decreased at the cell surface, persisting until at least 4 hours post-tetanus (Williams et al., 2007). These results may indicate a shift in receptor subunit composition following synaptic activity, such that synaptic priority is given to GluA1-containing AMPAR.

Previously, we have shown that the trafficking of GluA1-containing AMPAR, synaptic protein synthesis, and expression of Arc protein is dependent on the activity of PKG and MAPK (Claasen et al., 2009; Mockett et al., 2011; Livingstone et al., 2019), and may involve cGMP (Ishida et al., 1997). Interestingly, the NO-cGMP-PKG and MAPK pathways have been found to be involved in the declustering of GluA2/3-containing AMPAR (Endo and Launey, 2003), an event which is typically associated with subsequent internalization of receptors (Matsuda et al., 2000), indicating that these processes may be linked through a similar mechanism. Additionally, while sAPPα treatment has been shown to reliably protect against Aβ-induced impairments in LTP (Morrissey et al., 2019b), spine morphology (Tackenberg and Nitsch, 2019), and memory (Tan et al., 2018), these impairments appear to be mediated through the removal of cell surface GluA3-containing AMPAR at the synapse (Reinders et al., 2016), and transgenic APP/PS1 mice lacking GluA3 do not show increased mortality or memory deficit, like APP/PS1 mice. Here, the authors note that chronic downregulation of GluA3-containing AMPAR, without compensatory or activity-dependent increases in GluA1- or GluA2-containing AMPAR permit the disintegration of the synapse, ultimately leading to impairments in LTP and memory. This may suggest that sAPPα acts in one manner to protect neurons through the rapid and persistent internalization of GluA3-containing AMPAR and filling of these available slots with AMPAR that promote synaptic strengthening.

Importantly, it has been shown that the endocytosis of GluA2/3-containing AMPAR is Arc-dependent in cultured hippocampal slices (Rial Verde et al., 2006), and during conditioned morphine withdrawal (Liu et al., 2012). We have previously shown that the endocytosis of *de novo* GluA1-containing AMPAR is dependent on the synthesis of Arc protein (refer section 6.1.5. *Internalization of de novo GluA1 is Dependent on Arc Expression*), therefore it may also be possible that Arc has dual roles in targeting both GluA1- and GluA2/3-containing AMPAR. While we have provided evidence that *Arc* mRNA levels remained unchanged at 15- and 30-minute treatments, it is possible the early endocytosis of GluA2/3-containing AMPAR is mediated by the synthesis of Arc protein from local mRNA pools, independent of *de novo* transcription (Steward et al., 2015). Alternatively, endocytosis of GluA2/3-containing AMPAR may be under the influence of other endocytosis-related proteins, such as candidate plasticity gene 2 (CPG2), found to regulate both constitutive and LTD-dependent removal of GluA2-containing AMPAR from the cell surface (Loebrich et al., 2013; Cottrell et al., 2004).

7.4. *A Role for Arc Expression and Function*

Within the past 10 years, the immediate early gene Arc has been well characterised as a reliable and easily detectable marker of plasticity in both *in vitro* and *in vivo* applications (Link et al., 1995; Lyford et al., 1995; Minatohara et al., 2016). Arc expression is highly regulated, showing high signal-to-noise expression in both somatic and dendritic compartments following synaptic activity (Huang et al., 2007; Chawla et al., 2018; Janz et al., 2018). Here, we have shown that sAPPα treatment promotes the expression Arc mRNA and protein, in the soma and dendrites of cultured primary neurons. Notably, Arc expression was dependent on CaMKII, MAPK, and PKG activity, which is consistent with previous reports of signalling pathways which promote Arc expression during synaptic activity. Further these kinases have been shown to be essential for sAPPα's facilitation of local protein synthesis in hippocampal synaptoneurosomes and AMPAR trafficking in acute hippocampal slices (Claasen et al., 2009; Mockett et al., 2011). Additionally, we have identified a crucial relationship between NMDA and α7nAch receptors in triggering this effect. These findings provide evidence of a coordinated and overlapping set of signalling mechanisms through which sAPPα regulates the expression of plasticity-related proteins, including Arc, and thus regulates synaptic plasticity.

The regulation of Arc transcription and translation has been shown to be highly regulated, such that it has been hypothesized that there may exist separate pools of translating and non-translating *Arc* mRNA (Steward et al., 2014). *Arc* mRNA may be located and translated within the soma and serve to regulate gene transcription (Korb et al., 2013), while separate, translationally repressed pool is

trafficked throughout the dendrites, to be translated at synapses locally (Link et al., 1995). A third pool may govern rapid transcription-independent translation of *Arc* mRNA already associated with polyribosomes throughout the dendrites (Bagni et al., 2000; Na et al., 2016).

Early experiments provided evidence that nanomolar amounts of sAPPα promote the expression of *Arc* and *Zif268* mRNA in a time-dependent manner (refer section 4.1.1. *sAPPα Facilitates an Increase in Arc and Zif268 mRNA Expression*). Importantly, this was corroborated though observations that sAPPα increases Arc protein expression in a concentration-dependent manner, such that both 0.1 nM and 1 nM treatments of sAPPα for 2 hours significantly increased Arc protein expression in the dendrites, however only 1 nM treatment of sAPPα significantly enhanced somatic Arc protein (refer section 4.1.2. *sAPPα Facilitates an Increase in Arc Protein Expression*). This may be explained by the relative abundance of receptors necessary for sAPPα's activity. As described in section 4.1.6. *Dendritic Arc Protein Expression is Dependent on Activation of NMDA- and α7nACh Receptors*, we have shown that the full complement of dendritic Arc protein expression is dependent on the dual activity of NMDAR and α7nAChR. While both α7nAChR (Klein and Yakel, 2006; Fayuk and Yakel, 2007; Pidoplichko et al., 2013) and NMDAR (Dodt et al., 1998; Köhr, 2006) have been shown to be expressed at both somatic and dendritic membranes, it has been shown that α7nAChR-dependent Ca^{2+} signals are significantly larger in the dendrites than in the soma (Fayuk and Yakel, 2007), while the density of NMDAR is much greater in dendritic spines than in dendritic shaft and somatic membrane (Köhr, 2006). Therefore, the required signals for Arc expression may be reduced in response to 0.1 nM sAPPα within the soma.

Importantly, both the somatic and dendritic expression of Arc protein was found to be dependent on both transcription and translation (refer section 4.1.4. *Arc Protein Expression is Transcription- and Translation-Dependent*), further validating the observed increase in *Arc* mRNA, and likewise an increase in *Zif268* mRNA. Together these data provide evidence to suggest that sAPPα enhances the *de novo* transcription, synthesis and dendritic localization of Arc.

Of note, the concentration-dependent effects appeared to be specific to the treatment of sAPPα, as the closely related APP metabolite sAPPβ did not show similar effects. While previous reports indicate that sAPPα is able to rescue morphological and plasticity-related deficits observed in APP and APP-like protein 2 (APPL2) knockout mice (Ring et al., 2007; Fol et al., 2016) and conditional APP/APPL2 NexCre knockdown mice (Li et al., 2010; Hick et al., 2015), sAPPβ is unable to ameliorate these deficits. sAPPβ differs from sAPPα by a 16 amino-acid truncation at the C-terminus (CTα16). Due to this sAPPβ adopts a completely different structure to sAPPα (Peters-Libeu et al., 2015). Interestingly, application of CTα16 alone has been found to enhance LTP to a similar degree as sAPPα, in a manner

dependent on protein synthesis and activation of α7nAchRs (Morrissey et al., 2019b). The preservation of these amino acids in the sAPPα protein, and the common mechanisms employed by sAPPα and CTα16 stress a unique ability of these amino acids in mediating these effects. Therefore, the physiological differences between sAPPα and sAPPβ may underlie a divergence in each molecules ability to bind appropriate receptors, initiating different signalling cascades between the two sAPP metabolites, further distinguishing their distinct biological functions. Interestingly, treatment of cultures with 1 nM sAPPβ significantly reduced basal Arc protein in the soma but remained unaffected in the dendrites. Both sAPPα and sAPPβ have been found to bind $GABA_B$ receptors at high concentrations (250 nM; Rice et al., 2019) and enhanced $GABA_B$ activity has been tied to a reduction of Arc protein (Terunuma et al., 2014). Thus, sAPPβ may negatively modulate the expression of Arc protein by activation or modulation of $GABA_B$ receptors.

While previous work has described a multitude of signalling pathways mediating sAPPα-induced neuroprotection and plasticity, of particular interest is sAPPα's ability to increase cGMP and MAPK activity (Furukawa et al., 1996; Gakhar-Koppole et al., 2008). Interestingly, in previous studies using isolated synapses, sAPPα has been show to enhance *de novo* protein synthesis in a manner partially dependent on CaMKII and MAPK, and fully depending on PKG (Claasen et al., 2009). Likewise, trafficking of GluA1-containing AMPA receptors to the cell surface requires CaMKII and PKG, as well as protein synthesis (Mockett et al., 2011). In the present experiments, inhibition of CaMKII significantly impaired both somatic and dendritic Arc expression, while inhibition of MAPK and PKG significantly reduced Arc expression in the dendrites alone (refer section 4.1.5. *Arc Protein Expression is Dependent on CaMKII/MAPK/PKG Signalling*). Thus CaMKII, MAPK, and PKG may mediate distinct aspects of sAPPα-induced functions throughout each neuronal compartment.

Indeed, evidence suggests that PKG may act to facilitate trafficking of Rab11-positive vesicles. Rab11 is a protein primarily associated with recycling endosomes, and can mediate anterograde trafficking from the trans-Golgi network and perinuclear endosome (Chen et al., 1998; Takahashi et al., 2003; Ang et al., 2004; Lock and Stow, 2005). This trafficking is achieved through close association with the Ca^{2+}-sensitive motor protein myosin Vb (Wang et al., 2008). Interestingly, PKG has been linked to the nitric oxide (NO)-dependent stimulation of anterograde trafficking of Rab11A-positive recycling endosomes (Zhai et al., 2017) and has been shown to directly bind Rab11B (Reger et al., 2014). Both Arc protein and mRNA colocalize with Rab11 (Wu et al., 2011) and inhibition of Rab11 activity impairs postsynaptic expression of Arc protein in Drosophila motor neurons (Ashley et al., 2018). Thus, PKG may play an important role in the activity-dependent transport of Arc-containing vesicles throughout the dendrites. Additionally, PKG has been previously shown to be necessary for

the induction and persistence of protein synthesis-dependent LTP (Son et al., 1998; Kleppisch et al., 2003; Liu et al., 2003; Paul et al., 2008). Specifically, Liu et al., (2003) have shown that application of the PKG inhibitor Rp-8-Cl-cAMPS 5 minutes prior to, and 5 minutes following the induction of LTP by TBS, significantly attenuated the induction of LTP and fully eliminated its persistence. This may be explained through the PKG-dependent trafficking of GluA1 and the incorporation of CP-AMPAR at the synapse (Incontro et al., 2013) and the induction of IEGs, including Arc (Ota et al., 2010; Gallo and Iadecola, 2011). Therefore, it may be possible that PKG signals both the early insertion of CP-AMPAR as well as the trafficking of Arc protein, necessary for the conversion of early- to late-phase LTP (Plath et al., 2006).

The role of CaMKII and the regulation of Arc is intriguing. Arc protein is expressed in glutamatergic CaMKII-positive neurons and interacts with both CaMKIIα and CaMKIIβ to varying degrees. The interaction between CaMKIIα has been shown to be weaker than that of CaMKIIβ, however is able to regulate neurite extension (Donai et al., 2003). Interestingly, Arc protein shows stronger correlations with the inactive form of CaMKIIβ at inactive synapses following BDNF application *in vitro* or in the primary visual cortex *in vivo* (Okuno et al., 2012). While BDNF treatment alone was found to increase cell surface GluA1 expression, following BDNF treatment with the synapse specific application of the sodium channel blocker tetrodotoxin resulted in a downscaling of AMPAR at inactive synapses specifically (Okuno et al., 2012). These results indicate that while Arc is upregulated strongly with synaptic activity, and appears to localise to active synapses, preferential targeting to inactive synapses may govern control of Arc actions. In this sense, Arc may elicit non-Hebbian 'inverse synaptic-tagging' to maintain differences between synaptic weights during Hebbian plasticity. Interestingly, single synapse inactivation, as utilized by Okuno et al., (2012), has also been shown to promote synapse specific upscaling of CP-AMPAR in a manner dependent on the expression of Arc protein, however on a much longer timescale of hours to days (Béïque et al., 2011). Therefore, it may be possible that Arc acts as an inverse tag during synaptic activity at inactive sites in order to produce a reserve pool of GluA1-containing AMPAR to be utilized during synaptic upscaling following longer periods of activity.

Our results show that inhibition of CaMKII inhibits both dendritic as well as somatic Arc protein expression. In many cases, the expression of Arc is under control of CREB expression (Ying et al., 2002; Lv et al., 2015; Chen et al., 2017). In the current studies, we have shown that sAPPα enhances Arc protein expression in area CA1 of acute hippocampal tissue and within the dendrites of cultured hippocampal neurons, in a manner dependent on NMDAR and α7nAchR activation. Importantly, this increase occurs subsequent to an increase in pCREB (refer 4.2. *sAPPα Increases CREB Phosphorylation and Arc Protein in Acute Hippocampal Slices*). The Arc gene contains a binding site for

CREB protein located within a unique cluster of synaptic activity-responsive elements (SARE; Kawashima et al., 2009). Importantly, PKG (Gudi et al., 1999; Chen et al., 2003) and CaMKII have been shown to regulate CREB-dependent transcription or *Arc* mRNA transcripts (Sheng et al., 1991; Wheeler et al., 2008; Wheeler et al., 2012; Yan et al., 2016), with CaMKII likely mediating CREB phosphorylation via a mechanism which requires retrograde synapse to nucleus signalling (Ma et al., 2014a). Alternatively, once present at the synapse, PKG, in concert with MAPK (Huang et al., 2007; Bramham et al., 2010) and CaMKII may also regulate protein synthesis of synaptic proteins including Arc (Kleppisch et al., 2003; Claasen et al., 2009; Mockett et al., 2011), in a manner which may include direct phosphorylation of p38 MAPK (Browning et al., 2000; Michel et al., 2011) or CPEB (Atkins et al., 2004).

A crucial, yet outstanding question in the literature is the identity of the sAPPα receptor or receptors through which sAPPα may act to promote these downstream mechanisms. Previous evidence has described a role of $GABA_B$ (Rice et al., 2017; Rice et al., 2019), Na^+/K^+ ATP'ase (Dorard et al., 2018), α7nAch (Richter et al., 2018) and NMDA receptors (Gakhar-Koppole et al., 2008; Mockett et al., 2019) in mediating sAPPα's neurotrophic and plasticity-enhancing effects. To understand the receptors contributing to the facilitated Arc expression, we investigated the possible contributions of these and other candidate receptors, including mGluRI/II and TrkB, due to their similar neuromodulatory or plasticity-promoting properties (Raymond et al., 2000; Mockett et al., 2011). Of note, is the observed lack of dependence of TrkB, the receptor responsible for mediating the plasticity-enhancing effects of BDNF (Minichiello et al., 1999), indicating that the enhancement of Arc protein by sAPPα is distinct from that of BDNF.

Importantly, our data suggest a synergistic effect between activation of NMDARs and α7nAChRs (refer section 4.1.6. *Dendritic Arc Protein Expression is Dependent on Activation of NMDA- and α7nACh Receptors*). Sole inhibition of either receptor alone led to a partial impairment in the sAPPα-mediated expression of Arc protein, whereas simultaneous inhibition of both receptors fully eliminated the enhancement of Arc protein expression in the dendrites. Further, we have extended our studies to the more complex biological system of acute hippocampal slices (refer section 4.2. *sAPPα Increases CREB Phosphorylation and Arc Protein in Acute Hippocampal Slices*). Here, our findings closely reflected that of the primary hippocampal culture work, as sAPPα significantly enhanced Arc protein expression in area CA1 of the hippocampus in a manner dependent on activation of NMDA and α7nACh receptors. This finding was corroborated by an increase in pCREB expression. Interestingly, this effect was specific to area CA1, as likewise analysis of area CA3 showed

no significant change in Arc protein expression, while a small but non-significant increase in both pCREB and Arc protein was observed in the dentate gyrus.

NMDA and α7nACh receptors show somatic and dendritic distribution throughout hippocampal pyramidal neurons (Dominguez del Toro et al., 1994; Rao and Craig, 1997), and in some cases form α7nAChR-NMDAR complexes (Li et al., 2012; Li et al., 2013). Importantly, the activation of α7nAChR has been shown to enhance the activation of NMDAR (Aramakis and Metherate, 1998; Aramakis et al., 2000), specifically at synapses lacking functional AMPAR expression, likely through greater permeability to Ca^{2+} than that of NMDAR (McGehee, 1999; Levy and Aoki, 2002). Such α7nAChR-enriched, AMPAR-lacking synapses may depend on α7nAChR to provide voltage dependent activation of NMDAR. Importantly, both NMDAR and α7nAChR promote the activation of the NO-cGMP-PKG pathway (Contestabile, 2000; Serulle et al., 2007; Ota et al., 2010; Khan et al., 2016; Gulisano et al., 2019; Kusuda et al., 2020), as well as MAPK (Haddad, 2005; Gubbins et al., 2010; Yang et al., 2014; Ma et al., 2018) and CaMKII (Cammarota et al., 2000; Thalhammer et al., 2006; Gubbins et al., 2010), leading to the phosphorylation of CREB (Xia et al., 1996; Gubbins et al., 2010). Therefore, in order to transduce these signalling cascades, sAPPα may directly bind to these receptors. APP has been shown to co-immunoprecipitate with NMDAR subunits (Cousins et al., 2009; Innocent et al., 2012) and is found present at synapses expressing α7nAChR (Li et al., 2010), while sAPPα itself has been proven to act as a high-affinity, allosteric potentiator of α7nAChR (Forest et al., 2018; Richter et al., 2018). Importantly, Richter et al., (2018) have shown that application of the α7nAChR antagonist α-bungarotoxin (αBGT) following the application of sAPPα does not significantly affect the expression of sAPPα-enhanced LTP. However, co-application of sAPPα and αBGT before the induction of LTP significantly inhibits LTP expression. Importantly, these results indicate that sAPPα requires α7nAChR during the priming phase before the induction of LTP as α7nAChR do not appear to contribute directly to the induction or maintenance of LTP, likely indicating that α7nAChR activate downstream cascades and mechanisms which subsequently enhance LTP following TBS.

Many of the reports describing sAPPα's ability to enhance LTP have been shown in area CA1, including the dependence on NMDA (Mockett et al., 2019) and α7nACh receptors (Richter et al., 2018). Previous work has also shown that sAPPα enhances LTP within the dentate gyrus *in vivo*, however only occurring at concentrations no lower than 11 nM (Taylor et al., 2008), indicating that concentrations used in the current experiments may not be sufficient to drive the full complement of Arc expression. The dependence on NMDAR and α7nAChR in our primary hippocampal cultures, but the observed lack of effect within the dentate gyrus (refer section 4.2.1. *Trend in CREB Phosphorylation and Arc Protein Expression in the Dentate Gyrus of Acute Hippocampal Slices*) may in

fact be explained by the heterogeneity of our cultured neuron populations. While excitatory pyramidal neurons contain both NMDAR and α7nAChR (Perouansky and Yaari, 1993; Chung et al., 2016), excitatory granule cells appear to only express NMDARs (Bernabeu, 2000; Dalby and Mody, 2003; Wright and Jackson, 2014), and lack functional α7nACh receptors (Frazier et al., 2003; John et al., 2015). Interestingly, diverse populations of inhibitory neurons in CA1 and dentate gyrus subregions of the hippocampus contain both functional NMDAR and α7nAChR (McQuiston and Madison, 1999; Son and Winzer-Serhan, 2008; Carlén et al., 2012). In response to activity, Arc mRNA and protein expression has been noted within inhibitory neurons of both the hippocampus and cortex (Vazdarjanova et al., 2006; Wang et al., 2016). Therefore, within the mixed population of neurons analysed in our data set, it is possible that inhibition of NMDAR and α7nAChR during sAPPα treatment differentially targets distinct, but likely overlapping populations of dentate gyrus and CA-derived excitatory and inhibitory neurons expressing NMDAR, α7nAChR, or both. This interpretation may also be applied to acute hippocampal slices, such that sAPPα treatment may enhance Arc expression in both excitatory and inhibitory cells within area CA1, yet only affect interneurons within the dentate gyrus. Future experiments should aim to incorporate markers of inhibitory and excitatory neurons to aid analysis and further clarify the possible cell-type specific effects of sAPPα.

Alternatively, additional receptors may be required for the full complement of Arc protein expression throughout the hippocampus. Previously, sAPPα has been shown to enhance the expression and activity of Na^+/K^+ ATP'ase (Dorard et al., 2018). Na^+/K^+ ATP'ase is a membrane-bound enzyme responsible for generating and maintaining the Na^+ and K^+ electrochemical gradients across the cell membrane. Importantly, Na^+/K^+ ATP'ase is found throughout the hippocampus, including in CA pyramidal and interneuron cells (Richards et al., 2007), as well as excitatory and inhibitory cells of the dentate gyrus (Ross and Soltes, 2000). While research has yet to provide a link between Na^+/K^+ ATP'ase activity and Arc protein expression, the activation of NMDAR- dependent cGMP-NO-PKG signalling has been previously linked to the regulation of Na^+/K^+ ATP'ase (Munhoz et al., 2005), and the activation and expression of Na^+/K^+ ATP'ase regulates MAPK and CaMK activity, CREB phosphorylation, and may regulate aspects of LTP persistence (Gloor, 1997; Glushchenko and Izvarina, 1997; Desfrere et al., 2009). Interestingly, Na^+/K^+ ATP'ase has been found to associate with GluA1- and GluA2/3-containing AMPAR, and inhibition of Na^+/K^+ ATP'ase suppresses EPSPs and downregulates cell surface GluA1 and GluA2/3, possibly implicating this surface-bound enzyme in the regulation of AMPAR trafficking or sequestering at the cell surface (Zhang et al., 2009).

Regardless, these results indicate that concerted activity between NMDARs and α7nAChRs may act to promote sAPPα-mediated Arc expression through the synergistic activation of downstream

cascades involving CaMKII, PKG and MAPK. This activation of Arc protein may further regulate gene expression, expression of synaptic AMPAR, or modification of the actin cytoskeleton.

7.4.1. *Arc regulates de novo GluA1 expression*

Unexpectedly, short (30 min) but not long (120 min) treatments of sAPPα increased cell surface *de novo* GluA1, with expression of these AMPAR showed a return of *de novo* GluA1 to basal levels, at both the somatic and dendritic cell surface. Given past observations that surface internalisation under basal conditions is low (Passafaro et al., 2001), it is unlikely this is governed by a mechanism of passive endocytosis. Interestingly, clathrin-mediated endocytosis has been found capable of internalizing roughly 58.9% of AMPAR within 1-hour following acute insulin treatment (Man et al., 2000), placing a similar mechanism within our treatment window. Therefore, we further hypothesised that this decrease of *de novo* GluA1-containing AMPAR may be due to processes of active endocytosis. To examine this, we employed Acell™ siRNA-mediated inhibition of *de novo* Arc synthesis and protein expression (refer section 6.1.4. *Accell™ Arc siRNA Inhibits Somatic and Dendritic sAPPα-Dependent, but not Basal, Arc Expression*). Here, we found an accumulation and persistence of *de novo* GluA1-containing AMPAR following 2-hour co-incubation of siRNA with sAPPα (refer section 6.1.5. *Internalization of de novo GluA1 is Dependent on Arc Expression*), indicating that these AMPAR are internalised by Arc within the 2-hour treatment window, and inhibition of Arc expression prevents the observed reduction in these early expressed GluA1.

Previous work has provided evidence that Arc expression may govern regulation of GluA1-containing AMPAR. Knockout of Arc protein in primary hippocampal cultures has been shown to increase cell surface GluA1- but not GluA2-containing AMPAR, and occlude activity-dependent homeostatic scaling (Shepherd et al., 2006). Knockout of Arc expression *in vivo* shows a largely enhanced fEPSP during the initial 60 minutes following tetanisation, perhaps indicating an unregulated increase in CP-AMPAR at the synapse (Plath et al., 2006). Interestingly, this group found no increase in rectification during baseline stimulation, however this may be due to the activity-dependent nature of CP-AMPAR insertion and thus may have only been uncovered had rectification been measured post-tetanisation or at extrasynaptic sites. Interestingly, knockout of Arc in the visual cortex further upregulates s831 phosphorylation on the GluA1 AMPAR subunit (Gao et al., 2010), a phosphorylation site likely involved in the regulation of CP-AMPAR (Kim and Ziff, 2014; Yang et al., 2018; Park et al., 2019b; Summers et al., 2019). While these animals did not show enhanced cell surface GluA1 or GluA2 protein from total PSD homogenate, they did show enhanced mEPSC amplitude and impaired experience-dependent homeostatic plasticity. While enhancements in s831

phosphorylation did not alter rectification, this may indicate that absence of Arc protein leads to increased persistence of CP-AMPAR at the perisynaptic or extrasynaptic cell surface, and that this persistence occludes experience-dependent scaling. Further, in response to mGluR-mediated LTD, application of the mGluR agonist DHPG results in a decrease in rectification, alongside a decrease in cell surface GluA1 but not GluA2 protein. Given mGluR-LTD requires an increase in Arc protein synthesis (Waung et al., 2008) and removal of cell surface CP-AMPAR (Lanté et al., 2011; Scheyer et al., 2018), it is possible that this mechanism is governed through the actions of Arc. Conversely, overexpression of Arc protein has been previously shown to significantly increase the decrease rectification in neurons, in a manner effect dependent on Arc's ability to bind the clathrin-associated protein AP2 (DaSilva et al., 2016). These observations likely support a role for Arc-dependent endocytosis in the regulation of *de novo* CP-AMPAR in the current experiments. The Arc-dependent removal of these AMPAR may thus facilitate the exchange for GluA1/2-containing AMPAR by increasing the availability of synaptic slots (McCormack et al., 2006).

Additionally, we have shown that the *de novo* GluA1-containing AMPAR are primarily extrasynaptic (refer section 6.1.2.1. *sAPPα Enhances the Extrasynaptic, but not the Synaptic Population of de novo Cell Surface GluA1*). This domain contains proteins necessary for the formation of the endocytic zone and clathrin-coated pits (Lu et al., 2007a). Therefore, this may indicate this may indicate that *de novo* AMPAR present at extrasynaptic domains are primed to be removed from the cell surface following treatment of cultures with sAPPα for 30 minutes. Previously, it has been shown that extrasynaptic AMPAR are removed rapidly from the cell surface following NMDAR activation, and preceded the removal of synaptic AMPAR (Ashby et al., 2004; Sanderson et al., 2011). In these experiments, extrasynaptic sites showed a recovery of AMPAR following NMDAR washout, indicating that extrasynaptic sites are repopulated with either synaptic AMAPR from adjacent regions of the plasma membrane, or rapid trafficking of AMPAR from internal recycling pools. This may indicate that extrasynaptic AMPAR are initially internalized to facilitate the removal of synaptic AMPAR, possibly through the formation of extrasynaptic 'slots' within the endocytic zone (Haucke et al., 2011). This likely promotes the return of AMPAR to an internal recycling pool to be utilized in subsequent plasticity events. Interestingly, past evidence has shown that application of NMDA to cortical cultures transiently increases the phosphorylation of GluA1 at s831, followed by the dephosphorylation of s845 and removal of cell surface GluA1. This was found to increase the endosomal pool of GluA1-containing AMPAR and facilitate their reinsertion at the cell surface and later accumulation at synapses following both basal- and activity-dependent activity in primary cultures (Ehlers, 2000). Interestingly, BDNF has been shown to likewise regulate the expression of cell surface GluA1-containing AMPAR, such that short-term (30 minute) BDNF treatments enhance cell surface AMPAR in

nucleus accumbens medium spiny neurons, while long-term (24 hour) treatments decrease cell surface AMPAR beyond control levels These results indicate that homeostatic processes may govern the regulation of AMPAR expression following both BDNF and sAPPα treatments. The current experiments may reflect similar mechanisms, such that treatment of hippocampal neurons with sAPPα may transiently enhance the cell surface expression of CP-AMPAR and s831 phosphorylation, while Arc-dependent internalization of these AMPAR may act increase the endosomal recycling pool. The current experiment did not detect a decrease in total cell surface GluA1, as assessed by BioPLAy, however, examining the *de novo* population of GluA1-containing AMPAR with FUNCAT-PLA provided evidence for this.

7.5. *Mechanism of Action*

So far, this chapter has aimed to assess the role of sAPPα in regards to existing literature, encompassing aspects of LTP and synapse strengthening by previously identified neurotrophins. In an attempt to further clarify how this literature has influenced how sAPPα regulates the synthesis, expression, and function of AMPAR and Arc, two likely mechanisms are posited.

Firstly, the activation of NMDA and α7nACh receptors by sAPPα may promote NO, and cGMP signalling via Ca^{2+} influx, further promoting the activation of CaMKII, MAPK and PKG (Figure 7-1). These signalling cascades signal CREB phosphorylation and begin to enhance transcription of IEGs *Arc* and *Zif268*, and further upregulate miR-30 expression. During this time CAMKII, possibly in concert with PKG may promote the trafficking of pre-existing internal reserve pools of GluA1-containing CP-AMPAR to the cell surface, and likely stimulate lateral movement of AMPAR from extrasynaptic to perisynaptic sites. Simultaneously, NMDAR and α7nACh signalling may promote both the removal of GluA2/3-containing AMPAR by endocytosis, increasing the number of available synaptic slots. These AMPAR may then be targeted towards either the lysosome for degradation or recycling endosomes. These signalling cascades further promote the synthesis of GluA1 subunits either locally or via trafficking from somatic ER, the formation of CP-AMPAR, and refilling of internal recycling endosome pools. Parallel to this, *de novo* GluA2 subunits may be restricted from ER exit via associations with PICK1. Alternatively, miR-30 expression may downregulate GluA2 expression by inhibiting synthesis or promoting mRNA degradation. Regardless of the mechanism, a reduction in GluA2 forward trafficking promotes the formation of GluA1 homomeric CP-AMPAR. Following TBS, perisynaptic CP-AMPAR are inserted into the synapse to potentiate the EPSP, while extrasynaptic CP-AMPAR translocate to perisynaptic domains. During this time, *de novo* AMPAR likely traffic to the cell surface to expand the extrasynaptic pool, possibly governed via signals from synaptic CP-AMPAR. By 2 hours, synaptic CP-AMPAR may then diffuse back to the extrasynaptic membrane, and alongside *de novo* GluA1-

containing AMPAR, are removed from the cell surface, possibly by an Arc-dependent mechanism. Internalization of CP-AMPAR may permit the trafficking and replacement with GluA1/2-containing AMPAR at synaptic or extrasynaptic sites.

Alternatively, in addition to promoting the lateral trafficking of pre-existing cell surface CP-AMPAR, activation of NMDAR and α7nAChR may signal the rapid synthesis and exocytosis of *de novo* CP-AMPAR (Figure 7-2). Simultaneously, sAPPα promotes the removal of GluA2/3-contaning AMPAR, increasing the number of available synaptic slots. Following TBS stimulation in slices, these AMPAR may traffic to the synapse to enhance the EPSP and promote downstream signalling, returning to extrasynaptic sites to be removed during the maintenance of LTP. In culture, basal synaptic transmission may be sufficient to drive these AMPAR to the surface but not to the synapse (Oh et al., 2006), thus these AMPAR may reside at extrasynaptic sites. Regardless of whether *de novo* GluA1-containing AMPAR reach the synapse both pre-existing and *de novo* AMPAR return to, or reside at extrasynaptic sites, where Arc-dependent clathrin-mediated endocytosis occurs (Blanpied et al., 2002; Ashby et al., 2004; Tao-Cheng et al., 2011). The removal of these AMPAR likely permits the replacement of CP-AMPAR by GluA1/2-containing AMPAR.

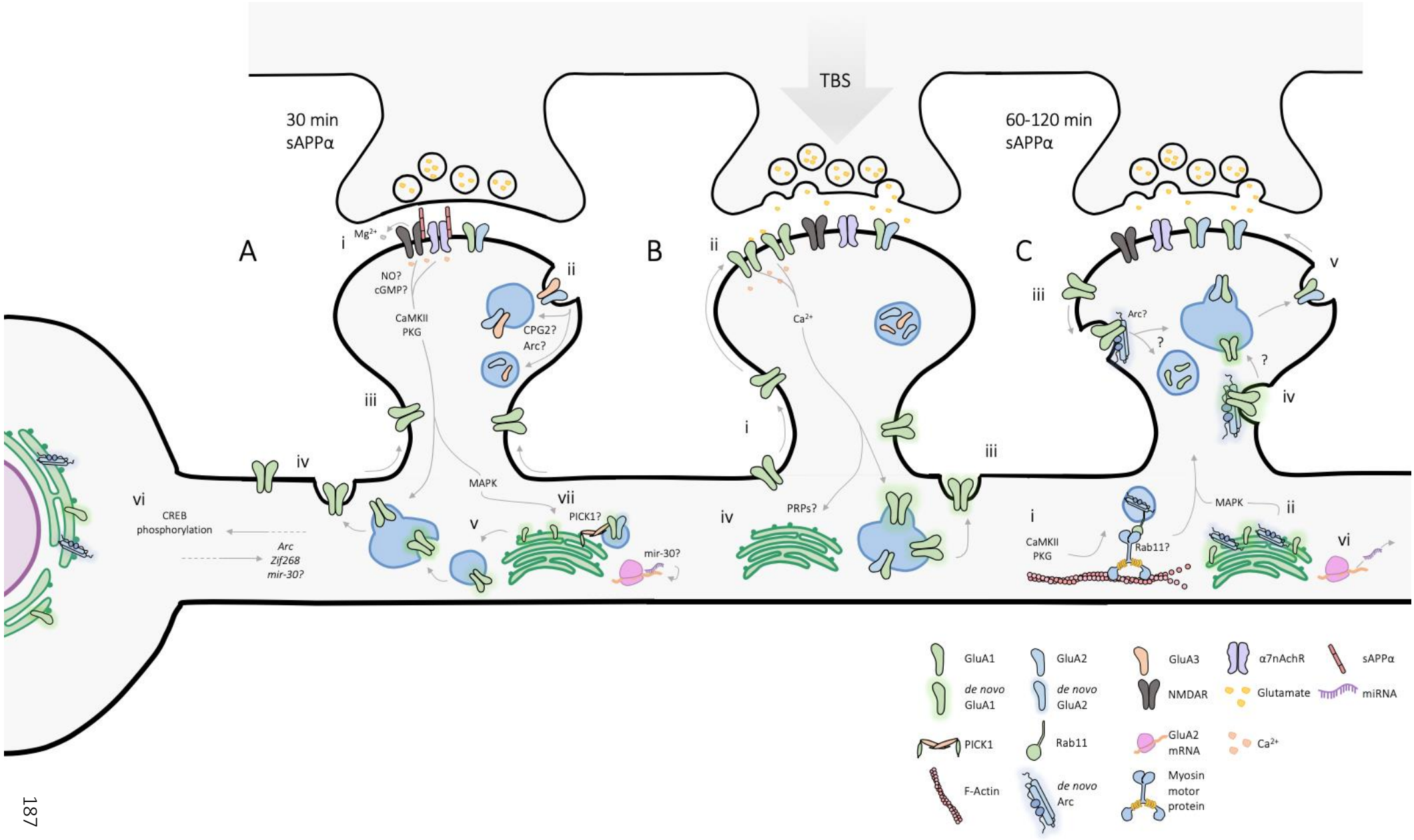

TBS
30 min
sAPPα
60-120 min
sAPPα
A
B
C
i
ii
iii
iv
v
vi
vii
Mg2+
NO?
cGMP?
CaMKII
PKG
CPG2?
Arc?
MAPK
PICK1?
mir-30?
CREB
phosphorylation
Arc
Zif268
mir-30?
Ca2+
PRPs?
Arc?
?
CaMKII
PKG
Rab11?
GluA1
de novo
GluA1
GluA2
de novo
GluA2
GluA3
NMDAR
α7nAchR
Glutamate
sAPPα
miRNA
PICK1
Rab11
GluA2
mRNA
Ca2+
F-Actin
de novo
Arc
Myosin
motor
protein

Figure 7-1 | Regulation of cell surface AMPAR – Extrasynaptic Exocytosis model. A) Application of sAPPα promotes the i) activation of NMDAR and α7nAchR, allowing the influx of Ca^{2+}, possible activation of NO and cGMP, and activation of CaMKII, MAPK, and PKG. Following this ii) GluA2/3-containing AMPAR are removed from the cell surface, possibly in a manner dependent on Arc or CPG2. These AMPAR are then targeted towards either the lysosome for degradation or recycling endosomes. During this time, iii) CP-AMPAR are trafficked towards the synapse by lateral diffusion. In addition to this, iv) CP-AMPAR are trafficked from existing internal stores to refill extrasynaptic domains. Downstream signalling may further v) enhance the early synthesis of GluA1 subunits for formation of *de novo* CP-AMPAR and refilling of recycling pools. Signalling from NMDAR and α7nAchR vi) also promotes CREB phosphorylation and induction of *Arc, Zif268*, and possibly miR-30 transcripts. vii) sAPPα may further promote reductions in cell surface *de novo* GluA2 expression through associations with PICK1 or by suppression of translation by miR-30. B) Following synaptic activity, i) perisynaptic CP-AMPAR are incorporated at the synapse, while extrasynaptic CP-AMPAR translocate to perisynaptic domains, and ii) synaptic activity at synaptic CP-AMPAR may promote the iii) trafficking of *de novo* CP-AMPAR to extrasynaptic sites. iv) Additionally, activity through CP-AMPAR has been shown to promote the protein-synthesis component of LTP (Park, 2018; Park et al., 2018), and thus may provide the necessary signals for the synthesis of additional plasticity related proteins (PRPs). C) Within 2 hours of sAPPα treatment CREB signalling promotes the transcription of *Arc* mRNA and subsequent protein. i) Arc mRNA or protein is thus trafficked throughout the dendrites in a manner dependent on CaMKII, MAPK, and PKG. Alternatively, ii) Arc protein may be synthesised locally. iii) Synaptic CP-AMPAR, as well as extrasynaptic *de novo* CP-AMPAR are removed from the cell surface via Arc, and are either iv) directed towards the lysosome for degradation or more likely refill the recycling pool of AMPAR. The removal of these AMPAR likely permit v) the exocytosis of GluA1/2-containing AMPAR from internal stores. vi) Previous restrictions of cell surface *de novo* GluA2 by PICK1 or miR-30 may be relieved by this time.

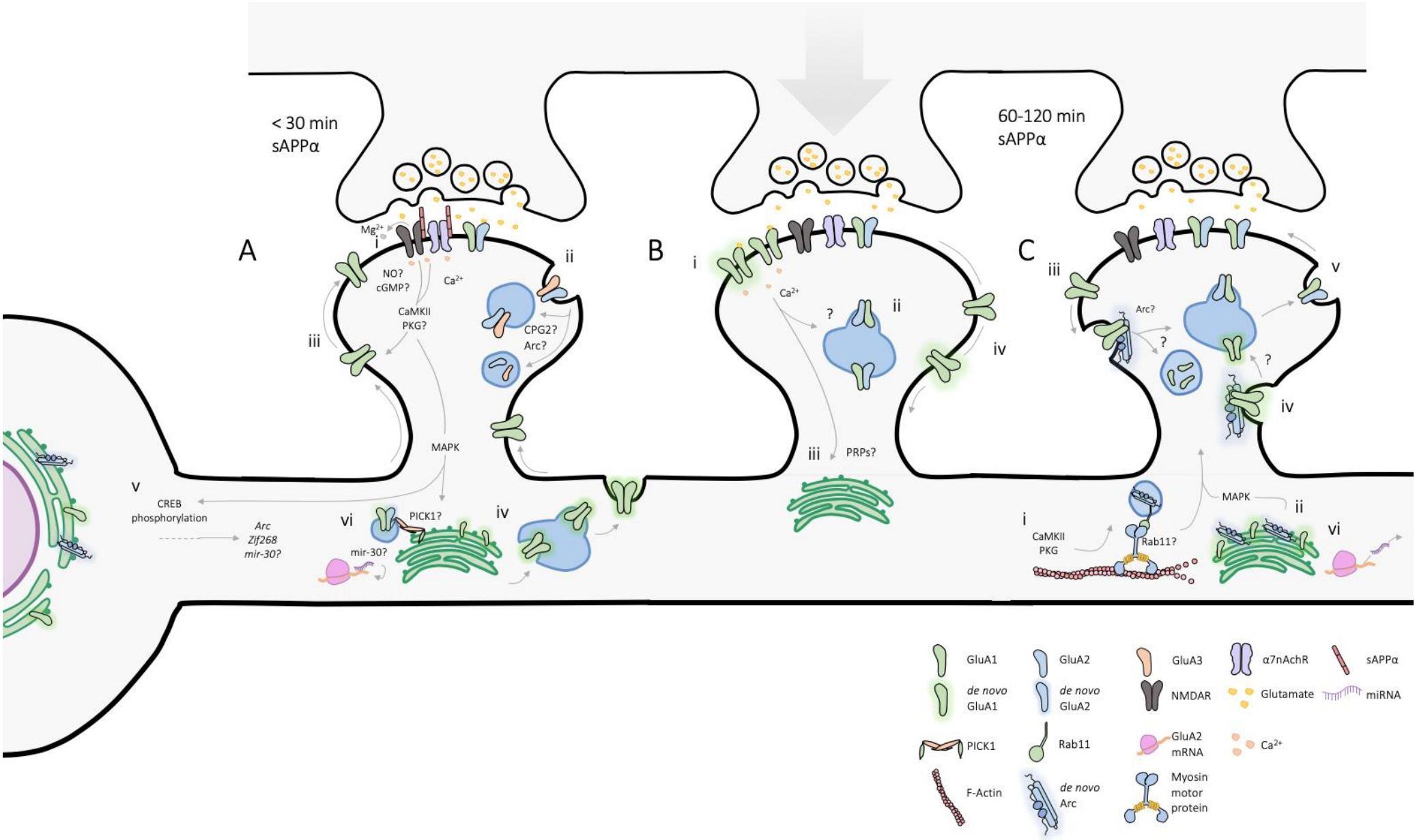

< 30 min
sAPPα
60-120 min
sAPPα
A
B
C
i
ii
iii
iv
v
vi
Mg^{2+}
NO?
cGMP?
Ca^{2+}
CaMKII
PKG?
CPG2?
Arc?
MAPK
CREB
phosphorylation
Arc
Zif268
mir-30?
PICK1?
mir-30?
PRPs?
Arc?
CaMKII
PKG
Rab11?
GluA1
de novo
GluA1
PICK1
F-Actin
GluA2
de novo
GluA2
Rab11
de novo
Arc
GluA3
NMDAR
GluA2
mRNA
Myosin
motor
protein
α7nAchR
Glutamate
Ca^{2+}
sAPPα
miRNA

Figure 7-2 | Regulation of cell surface AMPAR – Extrasynaptic Endocytosis model. Application of sAPPα promotes the i) activation of NMDAR and α7nAchR, allowing the influx of Ca^{2+}, possible activation of NO and cGMP, and activation of CaMKII, MAPK, and PKG. Following this ii) GluA2/3-containing AMPAR are removed from the cell surface, possibly in a manner dependent on Arc or CPG2. These AMPAR are then targeted towards either the lysosome for degradation or recycling endosomes. During this time, iii) CP-AMPAR are trafficked towards perisynaptic domains synapse by lateral diffusion. Downstream signalling may further enhance iv) the early synthesis of GluA1 subunits for formation of *de novo* CP-AMPAR and trafficking to the cell surface to refill extrasynaptic domains. Signalling from NMDAR and α7nAchR v) also promotes CREB phosphorylation and induction of *Arc, Zif268*, and possibly miR-30 transcripts. vi) sAPPα may further promote reductions in cell surface *de novo* GluA2 expression through associations with PICK1 or by suppression of translation by miR-30. B) Following synaptic activity, i) perisynaptic CP-AMPAR are incorporated at the synapse and ii) synaptic activity at CP-AMPAR may promote the iii) trafficking of *de novo* CP-AMPAR to synaptic sites. iv) Activity though CP-AMPAR has been shown to promote the protein-synthesis component of LTP (Park et al., 2018), and thus may provide the necessary signals for the synthesis of additional plasticity related proteins (PRPs), or the formation of existing GluA1/2 endosomal pools. Following this, CP-AMPAR including both pre-existing and *de novo* AMPAR may iv) return to extrasynaptic sites for endocytosis. C) Following 2 hours of sAPPα treatment CREB signalling promotes the transcription of *Arc* mRNA and subsequent protein. i) Arc mRNA or protein is thus trafficked throughout the dendrites in a manner dependent on CaMKII, MAPK, and PKG. Alternatively, ii) Arc protein may be synthesised locally. iii) Extrasynaptic pre-existing CP-AMPAR, as well as iv) extrasynaptic *de novo* CP-AMPAR are removed from the cell surface via Arc, and are either directed towards the lysosome for degradation, or more likely to refill the recycling pool of AMPAR. The removal of these AMPAR likely permits v) the exocytosis of GluA1/2-containing AMPAR from internal stores. vi) Previous restrictions of cell surface *de novo* GluA2 by PICK1 or miR-30 may be relieved by this time.

7.6. *A Possible Role for Silent Synapses*

In early postnatal development, the expression of NMDAR precedes that of AMPAR at glutamatergic synapses, acquiring AMPAR throughout later development (Petralia et al., 1999). These early AMPAR-lacking synapses are termed 'AMPA-silent' or 'silent' synapses, and have been proposed as a likely mechanism underlying synaptogenesis during both development and learning-induced plasticity. The presence of NMDAR permits NMDAR-dependent LTP, assuming the voltage-dependent Mg^{2+} block of NMDAR is relieved either by direct depolarisation of the postsynaptic membrane (Isaac et al., 1995), or activation of non-AMPAR receptors (Smith et al., 2005; Lozada et al., 2012). Many mechanisms have been put forward as a means to determine how silenced synapses become 'unsilenced,' or active. The existence of silent synapses has previously been explained through both presynaptic and postsynaptic mechanisms, with presynaptic mechanisms describing a handicap of neurotransmitter release in response to action potentials, with LTP enhancing both the probability of release (Gasparini et al., 2000) or increases in neurotransmitter quanta (Kullmann and Nicoll, 1992). Alternatively, postsynaptic changes, including AMPAR trafficking have been proposed (Liao et al., 2001; Xiao et al., 2004; Abrahamsson et al., 2007; Busetto et al., 2008; Huupponen et al., 2016).

Importantly, many of these postsynaptic trafficking mechanisms implicate CP-AMPAR in mitigating the formation of intermediate 'AMPA-labile' synapses. Upon synapse awakening, these AMPARs are thought to be inserted at previously AMPA-silent synapses and promote the further insertion of CI-AMPAR. Conversely, this period of lability also means recently awakened synapses are susceptible to re-silencing through low frequency baseline stimulation or LTD (Xiao et al., 2004; Morita et al., 2014). The initial formation of AMPA-silent synapses appears to be under the guidance of CaMKIV and CREB, while the insertion of CP-AMPAR to AMPA-labile synapses has been shown to be dependent on s831 phosphorylation, CaMKII and PKA (Liao et al., 2001; Poncer et al., 2002; Zheng and Keifer, 2009). In the adult brain, few silent synapses exist at basal (Petralia et al., 1999), however, silent synapses have been found to exist within DIV 21-28 primary cell cultures (Liao et al., 1999; Xu et al., 2020). Notably, while synapses present on spines were primarily occupied by both AMPAR and NMDAR by DIV 11, many dendritic shaft synapses were AMPAR-lacking even at DIV 23. Importantly, following cLTP, many of these AMPAR-lacking synapses gained greater proportions of GluA1 than synapses present in spines, and thus were deemed more "potentiable" (Xu et al., 2020).

In vivo, many mechanisms have been shown which enhance both the formation of AMPA-silent and AMPA-labile synapses, as well as their conversion into mature synapses. Of note, cocaine-induced drug addiction behaviour has been shown to enhance the proportion of silent synapses in adult rats (Brown et al., 2011), initially through the synthesis and insertion of GluN2B-containing NMDAR receptors (Huang et al., 2009), followed by insertion of CP-AMPAR (Lee et al., 2013; Ma et al.,

2016; Wright et al., 2020), possibly in a manner involving dopamine- or BDNF-dependent local protein synthesis (Itami et al., 2003; Smith et al., 2005). Following the generation of silent synapses, withdrawal of cocaine or application of an enriched environment induces LTP-like mechanisms to enhance both the synthesis (Churchill et al., 1999) and synaptic expression (Boudreau and Wolf, 2005) of GluA1-containing AMPAR, in a manner which occludes additional LTP (Goto and Grace, 2005; Moussawi et al., 2009; Ma et al., 2016). Interestingly, application of the mGluR5 agonist DHPG during the CP-AMPAR mediated AMPA-labile period also induces a removal of CP-AMPAR and replacement of CI-AMPAR (McCutcheon et al., 2011), while application of NMDAR-dependent LTD re-silences synapses by the removal of CP-AMPAR without replacement (Ma et al., 2016). Many systems which regulate local protein synthesis and trafficking of CP-AMPAR also regulate the expression and development of silent synapses, including both dopamine and BDNF (Smith et al., 2005; Caldeira et al., 2007; Li and Wolf, 2011; Fortin et al., 2012; Leal et al., 2014). Specifically, BDNF has been shown to play an important role in the maturation of silent synapses (Itami et al., 2003). Knockout of BDNF shows an increase in the proportion of silent synapses and an inability to convert these synapses following an LTP pairing protocol. Application of exogenous BDNF restores the ability to convert silent synapses to mature synapses, requiring rapid GluA1-dependent trafficking, followed by a GluA2-dependent trafficking.

With relevance to the current studies, we posit that one of the possible mechanisms through sAPPα may regulate synaptic plasticity is, in part, through the formation or maturation of silent synapses. Previously in this chapter, we have provided evidence that sAPPα may modulate synaptic plasticity at mature synapses, however these mechanisms may not be mutually exclusive (Figure 7-3). sAPPα has been shown to enhance the expression (Hoe et al., 2009; Mockett et al., 2011) and activation (Xiong et al., 2004) of NR1- and NR2B- containing AMPAR, a necessary step in the induction of AMPA-silent synapses (Nakayama et al., 2005; Xia et al., 2017). In addition to this, we have shown that sAPPα enhances the early expression of *de novo* GluA1-containing CP-AMPAR (refer section 5.1.3. *CP-AMPAR Contribute to the Initial Enhancement of sAPPα-LTP* and 6.1.2*. sAPPα Promotes the Rapid and Transient Trafficking of de novo GluA1-containing AMPAR to the Somatic and Dendritic Cell Surface*), likely exchanging these with GluA2-containing AMPAR (refer section 6.1.7. *sAPPα Promotes the Delayed Expression of Cell Surface GluA1/2-Containing AMPAR*). In further support of this, we have also shown that Arc protein synthesis is, in part, regulated by α7nAchR as well as NMDAR (refer section 4.1.6. *Dendritic Arc Protein Expression is Dependent on Activation of NMDA- and α7nACh Receptors*), while others have shown that α7nAchR are necessary for sAPPα's enhancement of LTP (Richter et al., 2018). Importantly, α7nAchR are found to be present at both AMPAR-positive and

AMPAR-negative synapses (Levy and Aoki, 2002), but also couple to (Li et al., 2012; Zhang et al., 2016a) and regulate NMDAR (Delibas et al., 2005; Shen et al., 2016; Tang et al., 2018). It has been recently shown that nicotinic activation of α7nAchR enhances the frequency of postsynaptic AMPA-mediated mEPSCs, typically associated with a decrease in the proportion of AMPA-silent synapses, and an increase in AMPAR-responses (Isaac et al., 1995; Tang et al., 2015). This effect was found to be dependent on the trafficking of CP-AMPAR, as well as phosphorylation mediated by CaMKII, PKC, and PKA (Tang et al., 2015). These results possibly implicate α7nAchRs in mediating an enhancement of AMPA-labile synapses and therefore may play a role in enhancing synaptic plasticity in response to sAPPα treatment by a similar means.

Morphologically, the structural correlates of silent synapses are relatively unknown. Observations from cell culture indicate that AMPA-silent synapses exist primarily on the dendritic shaft (Xu et al., 2020), with NR2B-containing NMDAR appear to predominate in young spines, and an increasing contribution of GluA1 as the synapse matures (Oray et al., 2005). Cocaine- and alcohol-dependent addiction behaviour studies further indicate that the formation of silent synapses may occur through the generation of new spines, finding that the number of spines increases by increasing the proportion of new immature thin and filopodia spines, with no change in the number of mature mushroom spines (Graziane et al., 2016; Beroun et al., 2018). Conversely, silent synapses have also been observed to occur at mushroom spines (Busetto et al., 2008), concluding that the morphology of silent synapses does not always predict the AMPAR content. Additionally, this may indicate that rejuvenation or elimination of existing synaptic contacts also occurs by mechanisms governing the trafficking of AMPAR and the silencing and unsilencing of these synapses (Dong, 2016).

We have further shown that the proportion of *de novo* cell surface GluA1-containing AMPAR are expressed within extrasynaptic domains (refer section 6.1.2.1. *sAPPα Enhances the Extrasynaptic, but not Synaptic Population of de novo Cell Surface GluA1*). Interestingly, it has been previously shown that extrasynaptic CP-AMPAR permit the formation of new synapses, by providing the necessary NDMAR voltage-dependent relief (Schmidt-Salzmann et al., 2014). These synapses appear capable of transducing presynaptic activity and integrating into the neural circuit (Kwon and Sabatini, 2011). Further, CP-AMPAR have been shown to be essential for the formation of synapses following repetitive LTP in culture (Tominaga-Yoshino et al., 2020), a stimulation paradigm which enhances synaptogenesis, increasing the proportion of immature synapses and the frequency of miniature EPSCs (Urakubo et al., 2006; Tominaga-Yoshino et al., 2008). Therefore, the presence of extrasynaptic CP-AMPAR following sAPPα treatment may not be a passive mechanism, and may instead contribute significantly to the formation of new spines and synapses.

Lastly, APP, and specifically sAPPα have been implicated in the formation and maturation of synapses (Wang et al., 2009; Southam et al., 2019). Knockout of APP reduces spine density (Tyan et al., 2012), in a manner which is restored with viral expression of sAPPα, but not sAPPβ (Richter et al., 2018). This knockout of APP preferentially increases the number of stubby spines, with a relative decrease in mushroom spines, while knock-in of sAPPα restores the proportion of mushroom spines, with relative decreases in stubby spines (Weyer et al., 2014). These results indicate that chronic expression of sAPPα may promote the conversion of young, possibly AMPA-silent, spines into mature, functional spines. Conversely, the Aβ domain of APP has been found necessary for activity-induced synaptic depression (Kamenetz et al., 2003), and the reduction of synaptic GluA1, s831 phosphorylation, and CaMKII *in vitro,* further decreasing mEPSC frequency *ex vivo* (Gu et al., 2009). These characteristics were found to impair the conversion of silent synapses into functional synapses and correlated to impaired induction of LTP and performance on a spatial memory task (Bie et al., 2018). Together, this evidence suggests that sAPPα and Aβ may play opposing roles in the generation and maintenance of silent synapses.

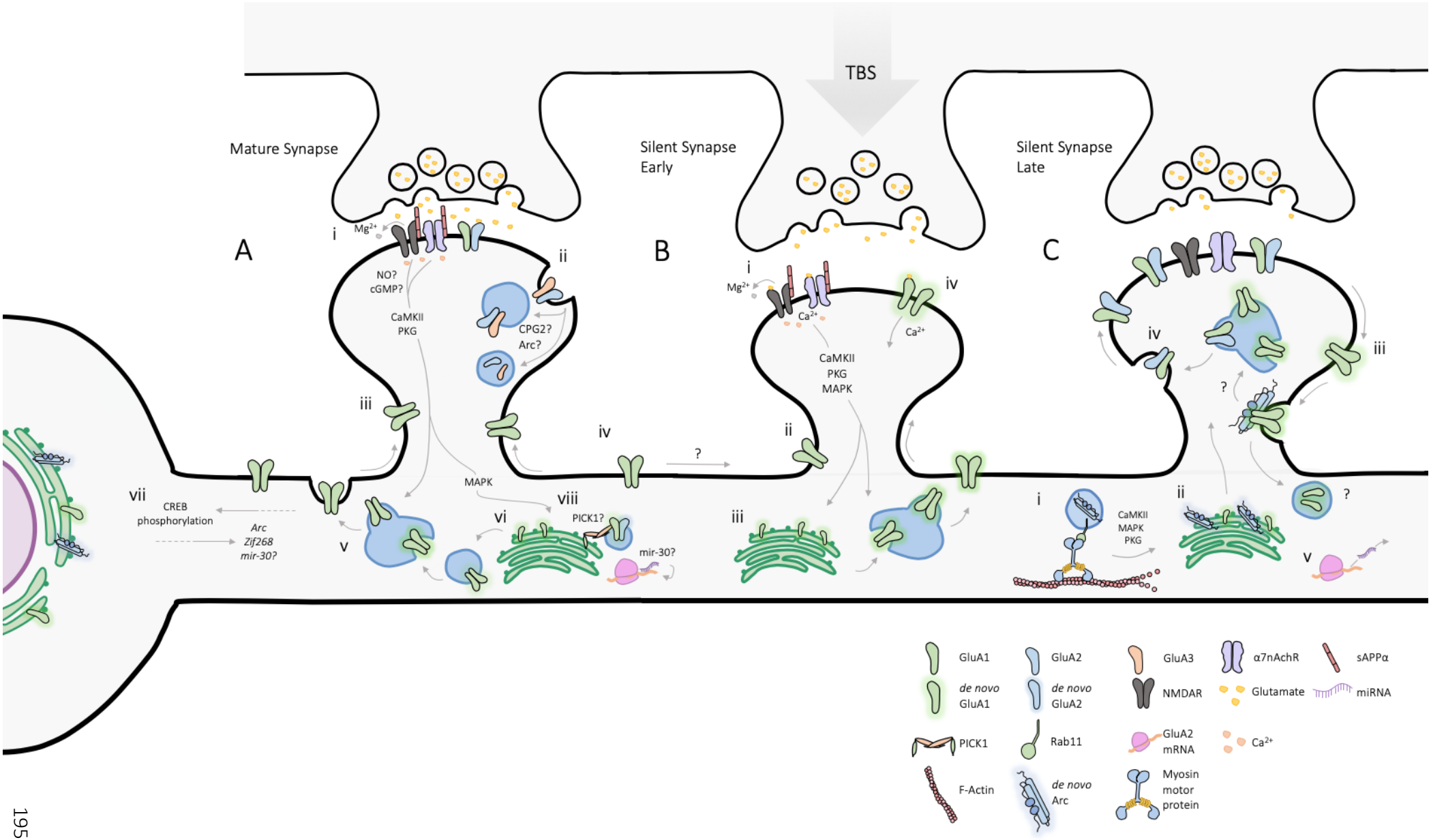

TBS
Mature Synapse
Silent Synapse
Early
Silent Synapse
Late
A
B
C
i
ii
iii
iv
v
vi
vii
viii
Mg2+
NO?
cGMP?
CaMKII
PKG
CPG2?
Arc?
MAPK
CREB
phosphorylation
Arc
Zif268
mir-30?
PICK1?
mir-30?
Ca2+
CaMKII
PKG
MAPK
?
GluA1
de novo
GluA1
PICK1
F-Actin
GluA2
de novo
GluA2
Rab11
de novo
Arc
GluA3
NMDAR
GluA2
mRNA
Myosin
motor
protein
α7nAchR
Glutamate
Ca2+
sAPPα
miRNA

Figure 7-3 | Regulation of cell surface AMPAR at silent synapses. A) Application of sAPPα promotes the i) activation of NMDAR and α7nAchR, allowing the influx of Ca^{2+}, possible activation of NO and cGMP, and activation of CaMKII and PKG. Following this, ii) GluA2/3-containing AMPAR are removed from the cell surface, possibly in a manner dependent on Arc or CPG2. These AMPAR are then targeted towards either the lysosome for degradation or recycling endosomes. During this time, iii) CP-AMPAR are trafficked towards the synapse by lateral diffusion (Ehlers et al., 2007). iv) This lateral diffusion may also supply the CP-AMPAR at silent synapses by diffusional trapping (Harrison et al., 1991; Ehlers et al., 2007). Alternatively, v) a pool of CP-AMPAR are trafficked to the cell surface by exocytosis. vi) MAPK signalling may then promote the rapid synthesis and refilling of these AMPAR at recycling pools. Signalling from NMDAR and α7nAchR vii) also promotes CREB phosphorylation and induction of *Arc, Zif268*, and possibly miR-30 transcripts. viii) sAPPα may further promote reductions in cell surface *de novo* GluA2 expression through associations with PICK1 or by suppression of translation by miR-30. B) At immature, AMPA-silent synapses, i) activation of NMDAR and α7nAchR promotes the activation of CaMKII, PKG, and MAPK signalling permitting the ii) trapping of cell surface AMPAR, supplied by neighbouring mature synapses, or iii) the synthesis and trafficking of *de novo* CP-AMPAR to the synapse, effectively converting a previously AMPA-silent synapses into a labile, awakened synapse. While it is possible that exocytosis of existing CP-AMPAR from recycling endosomes also occurs at silent synapses, this would likely only occur at synapses which had previously been unsilenced and have formed a local pool of endosomal AMPAR following silencing (Xiao et al., 2004). Alternatively, silent synapses may share endosomal compartments with neighbouring mature synapses, however, this appears rare (Cooney et al., 2002; English et al., 2015). Following synaptic activity these AMPAR may then integrate into the synapse and iv) further promote the influx of Ca^{2+} and downstream signalling cascades, as well as contribute to the EPSP. C) Within 2 hours of sAPPα treatment, CREB signalling promotes the transcription of *Arc* mRNA and subsequent protein. i) Arc mRNA or protein is thus trafficked throughout the dendrites in a manner dependent on CaMKII, MAPK, and PKG. Alternatively, ii) Arc protein may be synthesised locally. iii) Synaptic CP-AMPAR may be removed from the cell surface via Arc, and permit iv) the exocytosis of GluA1/2-containing AMPAR from internal stores. v) Previous restrictions of cell surface *de novo* GluA2 by PICK1 or miR-30 may be relieved by this time.

7.7. *A Promising Role for sAPPα in Synaptic Disorders*

Many molecules and pathways are involved in the expression of both basal synaptic transmission, the induction of synaptic plasticity, and the maintenance of synaptic strength. These processes are controlled through concerted actions of transcription factors (Alberini, 2009; Liu et al., 2011), the synthesis, degradation, and synaptic distribution of Arc and AMPA receptors (Cajigas et al., 2010; Korb and Finkbeiner, 2011; Park, 2018; Pandey et al., 2020), the actions of secreted factors, including neurotrophins, cytokines, hormones, and growth factors (Sastry et al., 1988; Kelly et al., 1998; Moult and Harvey, 2008; Wang et al., 2012), and the activation of signalling cascades by Ca^{2+}-sensitive kinases such as PKA, PKC, PKG, and CaMKII (Huang and Kandel, 1994; Wu et al., 2006; Giese and Mizuno, 2013).

Through this, research has aimed to target the modulation of these systems as a means towards manufacturing a therapeutic for neurodegenerative disorders including AD. Past research has focused on the delivery of neurotrophins and growth factors as a means to enhance downstream neuroprotective and neurotrophic signalling pathways (Aloe et al., 2012). However, the direct application of these molecules in clinical therapeutic use is limited, due to the insurmountable hurdles of unfavourable pharmacokinetic properties (plasma half-life <10 min; Wu, 2005), poor blood–brain barrier (BBB) permeability (Pardridge, 2002), and severe adverse effects (von Bartheld et al., 1994), including hyperinnervation of blood vessels (Isaacson et al., 1990), hypophagia (Williams, 1991), increased hyperexcitability and the susceptibility to seizures (Xu et al., 2004), and off-target effects (Zhao et al., 2017). Through this, alternative means of enhancing the endogenous production of neurotrophins and growth factors have arisen, including small-molecule mimetics (Kazim and Iqbal, 2016; Gupta and Sharma, 2017), botanicals (Sangiovanni et al., 2017), exercise (Rasmussen et al., 2009; Griffin et al., 2011; Coelho et al., 2014), dietary supplementation (Martin et al., 2017), and caloric restriction (Maswood et al., 2004). A facet of these treatments is the application of molecules which directly and positively modulate AMPAR function and kinetics directly. Research into these drugs, labelled AMPAkines, stems from the growing realization that abnormalities in synaptic plasticity contribute to a wide range of neurological and cognitive disorders, most notably AD, but extending to autism, bipolar disorder, schizophrenia, addiction, multiple sclerosis, Parkinson's disease and chronic pain (Bliss et al., 2013). AMPAkines include agonists (Black, 2005), positive allosteric modulators (Chappell and Witte, 2004), and treatments targeting signalling pathways regulating protein synthesis (Liu et al., 2015b), to facilitate AMPAR-mediated depolarization and mimic AMPAR insertion in LTP, increase depolarization-induced NMDAR Mg^{2+} relief, or enhance the induction of downstream neurotrophic factors to augment further signalling pathways. While some AMPAkines have shown promising results in clinical phase 2 studies in AD, Major Depressive Disorder (Bretin et

al., 2017), and Schizophrenia (Noorbala et al., 1999), they do so in the presence of side effects (Wezenberg et al., 2007; Urban and Gao, 2014). Due to this, research has begun to investigate alternative delivery methods of neurotrophins and growth factors into the CNS through means such as encapsulated cell bio-delivery devices (Eyjolfsdottir et al., 2016), viral vectors (Tuszynski et al., 2005), receptor-mediated BBB transcytosis (Pardridge et al., 1994), and biodegradable microspheres (Aubert-Pouëssel et al., 2004). Most recently, small (40-100 nm) lipid-membrane bound extracellular vesicles (EVs) have been studied as a means of targeted drug delivery (Ha et al., 2016; Akuma et al., 2019). These EVs, or exosomes, have the potential to package proteins of interest, as well as mRNA, cDNA, and miRNA (Statello et al., 2018), and are able to be delivered intranasally or intravenously (Haney et al., 2015), cross the BBB (Saint-Pol et al., 2020), and contain the specific expression of tetraspanins, which preferentially interact with ligands in a tissue- and cell- specific manner (Rana et al., 2012; Luan et al., 2017), thus decreasing off-target effects.

The neurotrophic, neuroprotective, and memory enhancing effects of sAPPα cannot go understated. Indirect modulators of α-secretase activity, including the GABAR modulator Etazolate (Marcade et al., 2008), the 5-HT4 agonist PRX-03140 (Sabbagh, 2009), the green tea extract epigallocatechin-gallate (Obregon et al., 2006), or the cholinesterase inhibitor Rivastigmine (Maccecchini et al., 2012) have shown promising alleviation of pathology-related symptoms. However, in order to prevent off-target side effects, cell- and tissue-targeted viral- or EV-mediated application of sAPPα, or equally effective peptides derived from sAPPα, should be considered the gold standard method of delivery, and warrant future research.

7.8. *Future Directions*

While the results described within this thesis support a novel role of sAPPα in the synthesis, trafficking, and expression of both the IEG Arc, as well as cell surface AMPA receptors, questions remain regarding the nature of these changes. Firstly, we have shown that sAPPα enhances the cell surface expression of *de novo* GluA1-containing AMPAR at extrasynaptic domains and their internalization parallels the expression of cell surface GluA1/2-containing AMPAR. In order to clarify the role of these AMPAR in sAPPα-mediated plasticity, experiments should aim to determine GluA1/2-containing AMPAR are synaptic, or likewise populate extrasynaptic sites. Additionally, due to sAPPα's ability to enhances local protein synthesis, further experiments should aim to address this with relevance to the synthesis of AMPAR or Arc protein. Microfluidic local perfusion chambers are a readily available alternative to MatTek culture dishes and allow for the compartmentalization of dendrites from the soma, as well as the ability to apply treatments to discrete populations of

synapses (Taylor et al., 2010). Further, recent advances allow for the photo-uncaging of puromycin, capable of labelling newly synthesised proteins with a higher degree of spatiotemporal resolution (Elamri et al., 2018). The use of these techniques would increase the spatial resolution of observed protein synthesis, as well as allow for much finer temporal quantification of protein synthesis occurring locally at synapses. Further, part of the hypothesis that sAPPα regulates cell surface AMPAR trafficking relies on the assumption that pre-existing or *de novo* cell surface AMPAR translocate towards the synapse by lateral diffusion (see Figure 7-1,Figure 7-2). In order to examine this, previous research has employed the cell surface labelling of AMPAR by nanosized fluorophores called quantum dots, for the examination of AMPAR lateral diffusion (Howarth et al., 2005). In addition to this, examining the contribution of AMPAR lateral diffusion in the enhancement of sAPPα-mediated LTP has been made possible through the cross-linking and immobilisation of cell surface AMPAR during the induction of LTP (Penn et al., 2017). These techniques used separately or combined would further enhance our understanding of the role of AMPAR trafficking and mobility in LTP following sAPPα treatment.

Secondly, throughout this thesis I have made comparisons to the neurotrophic protein BDNF and the neuromodulator dopamine, on the basis of observations from supporting literature. Like sAPPα, BDNF and dopamine enhance cell surface GluA1 (Bellone and Lüscher, 2006; Gao et al., 2006; Caldeira et al., 2007), and strengthen synaptic transmission (Ying et al., 2002; Shetty et al., 2017), however in a manner distinct from sAPPα. Therefore, in order to understand the full complement of sAPPα's neurotrophic support, and how these plasticity-enhancing proteins mediate the strengthening of synapses and formation of memories, biochemical and electrophysiological experiments should be made in order to compare the relative regulation of synaptic and cell surface AMPAR, contribution to LTP, and disparate or overlapping mechanisms of action.

Thirdly, as part of this thesis, we have proposed that sAPPα may act to strengthen synapses through the formation and awakening of AMPA-silent synapses. To further clarify this, examining the proportion of AMPAR-positive synapses by electron microscopy (Petralia et al., 1999) or the frequency of postsynaptic AMPA-mediated mEPSCs using *in vitro* by the use of multielectrode arrays (Arnold et al., 2005) or *ex vivo* field electrophysiology (Wasling et al., 2012), in the presence or absence of sAPPα, would be a worthy avenue to examine.

Finally, in order for sAPPα to express any relevant clinical applicability, the observations made within this thesis, as well as those previously described, including neuroprotection (Furukawa et al., 1996; Ryan et al., 2013; Tackenberg and Nitsch, 2019), neurogenesis (Demars et al., 2011), and morphological- and synaptic plasticity (Clarris et al., 1994; Taylor et al., 2008; Mockett et al., 2011), should be made through the use of cultured transgenic APP/PS1 mouse cultures as well as cultured

human neurons. As shown previously, the culturing of adult mouse neurons expressing mutations in APP/PS1 genes is possible (refer section 3.2. *Observations from the Culturing of Adult Cortical Mouse Neurons)*. In addition this, recent research has established both induced pluripotent stem cell (IPSC) cultures (Konagaya et al., 2015), as well as the direct culturing of patient derived neural tissue (Lee et al., 2020), for the incomparable advantage of a direct representation of human neurons in culture. Ideally, these systems would be used in conjunction with delivery methods such as the incorporation of sAPPα into exosomes or viruses for the justification of these delivery methods *in vivo*, and allowing for the analysis of cell-specific effects, justifying any future clinical applicability.

Many of the experiments and hypotheses described within this thesis examine the expression of AMPAR in primary hippocampal cultures. This system benefits from an increased spatial resolution to that of more complex biological systems, however limitations exist due to possible genetic (Bowling et al., 2016) and physiological (Szcot et al., 2010) differences between cultured neurons and acute hippocampal slices. It has recently been shown that FUNCAT-PLA is possible in *ex vivo* preparations (Evans et al., 2019), thus in combination with cell surface biotin-labelling techniques (Mockett et al., 2019), it is possible that total *de novo* AMPAR as well as cell surface *de novo* AMPAR could be targeted for analysis by immunohistochemistry and western blot, respectively. This would add strength to the conclusions drawn from cultured neurons by examining AMPAR expression in intact tissue.

7.9. *Concluding Remarks*

This research aimed to examine the role of Arc protein expression and AMPAR trafficking in hippocampal neurons. The experiments in this thesis support previous work indicating that sAPPα enhances protein synthesis and AMPA receptor trafficking. Here, we have shown that sAPPα enhances LTP in a manner dependent on the synaptic expression of Ca^{2+}-permeable GluA1-containing homomeric AMPAR. Further, we have shown that sAPPα enhances the expression of both pre-existing and newly synthesised GluA1 at the cell surface. Curiously, while an increase in GluA1 protein was found at the neuronal surface following both short-term and long-term treatments, the cell surface expression of *de novo* GluA1-containing AMPAR was short-lived, and found to be internalized from the cell surface in an Arc-dependent manner. Complementary to this, we have described a role for GluA1/2- and GluA2/3-containing AMPAR, finding a rapid and persistent decrease in cell surface GluA2/3-containing AMPAR specific to the dendrites, possibly as a means to increase available synaptic slots. Conversely, GluA1/2-containing AMPAR show a pattern of delayed cell surface expression and may act to replace homomeric GluA1-containing AMPAR.

The research described in this thesis describes a novel role of sAPPα in the regulation of key plasticity-related proteins, in line with similar neurotrophins, and describes sAPPα as a significant modulator of plasticity in hippocampal neurons. These observations further our understanding of synaptic plasticity in both health and disease and set a strong precedent for subsequent experiments regarding the functions and mechanisms of sAPPα.

References

Abbott, L.F., and Nelson, S.B. (2000). Synaptic plasticity: taming the beast. *Nat Neurosci* 3 Suppl, 1178-1183. doi: 10.1038/81453.

Abel, T., and Nguyen, P.V. (2008). Regulation of hippocampus-dependent memory by cyclic AMP-dependent protein kinase. *Prog Brain Res* 169, 97-115. doi: 10.1016/s0079-6123(07)00006-4.

Abel, T., Nguyen, P.V., Barad, M., Deuel, T.A.S., Kandel, E.R., and Bourtchouladze, R. (1997). Genetic Demonstration of a Role for PKA in the Late Phase of LTP and in Hippocampus-Based Long-Term Memory. *Cell* 88(5), 615-626. doi: https://doi.org/10.1016/S0092-8674(00)81904-2.

Abeliovich, A., Chen, C., Goda, Y., Silva, A.J., Stevens, C.F., and Tonegawa, S. (1993). Modified hippocampal long-term potentiation in PKC gamma-mutant mice. *Cell* 75(7), 1253-1262.

Abraham, W.C. (2003). How long will long-term potentiation last? *Philosophical Transactions of the Royal Society B: Biological Sciences* 358(1432), 735-744. doi: 10.1098/rstb.2002.1222.

Abraham, W.C. (2008). Metaplasticity: tuning synapses and networks for plasticity. *Nature Reviews Neuroscience* 9(5), 387-387. doi: 10.1038/nrn2356.

Abraham, W.C., and Bear, M.F. (1996). Metaplasticity: the plasticity of synaptic plasticity. *Trends Neurosci* 19(4), 126-130. doi: 10.1016/s0166-2236(96)80018-x.

Abraham, W.C., and Goddard, G.V. (1983). Asymmetric relationships between homosynaptic long-term potentiation and heterosynaptic long-term depression. *Nature* 305(5936), 717-719.

Abraham, W.C., and Williams, J.M. (2003). Properties and Mechanisms of LTP Maintenance. *The Neuroscientist* 9(6), 463-474. doi: 10.1177/1073858403259119.

Abraham, W.C., Logan, B., Greenwood, J.M., and Dragunow, M. (2002). Induction and experience-dependent consolidation of stable long-term potentiation lasting months in the hippocampus. *J Neurosci* 22(21), 9626-9634. doi: 10.1523/jneurosci.22-21-09626.2002.

Abrahamsson, T., Gustafsson, B., and Hanse, E. (2007). Reversible synaptic depression in developing rat CA3 CA1 synapses explained by a novel cycle of AMPA silencing-unsilencing. *J Neurophysiol* 98(5), 2604-2611. doi: 10.1152/jn.00602.2007.

Abramov, E., Dolev, I., Fogel, H., Ciccotosto, G.D., Ruff, E., and Slutsky, I. (2009). Amyloid-β as a positive endogenous regulator of release probability at hippocampal synapses. *Nature Neuroscience* 12, 1567. doi: 10.1038/nn.2433

Adesnik, H., and Nicoll, R.A. (2007). Conservation of glutamate receptor 2-containing AMPA receptors during long-term potentiation. *J Neurosci* 27(17), 4598-4602. doi: 10.1523/jneurosci.0325-07.2007.

Adesnik, H., Nicoll, R.A., and England, P.M. (2005). Photoinactivation of Native AMPA Receptors Reveals Their Real-Time Trafficking. *Neuron* 48(6), 977-985. doi: https://doi.org/10.1016/j.neuron.2005.11.030.

Agranoff, B.W. (1967). Memory and protein synthesis. *Sci Am* 216(6), 115-122.

Agranoff, B.W., and Klinger, P.D. (1964). Puromycin Effect on Memory Fixation in the Goldfish. *Science* 146(3646), 952-953. doi: 10.1126/science.146.3646.952.

Agranoff, B.W., Davis, R.E., and Brink, J.J. (1965). Memory fixation in the goldfish. *Proc Natl Acad Sci U S A* 54(3), 788-793. doi: 10.1073/pnas.54.3.788.

Ahi, J., Radulovic, J., and Spiess, J. (2004). The role of hippocampal signaling cascades in consolidation of fear memory. *Behavioural Brain Research* 149(1), 17-31. doi: https://doi.org/10.1016/S0166-4328(03)00207-9.

Ainsley, J.A., Drane, L., Jacobs, J., Kittelberger, K.A., and Reijmers, L.G. (2014). Functionally diverse dendritic mRNAs rapidly associate with ribosomes following a novel experience. *Nature Communications* 5(1), 4510. doi: 10.1038/ncomms5510.

Akaneya, Y., Tsumoto, T., Kinoshita, S., and Hatanaka, H. (1997). Brain-Derived Neurotrophic Factor Enhances Long-Term Potentiation in Rat Visual Cortex. *The Journal of Neuroscience* 17(17), 6707-6716. doi: 10.1523/jneurosci.17-17-06707.1997.

Akhtar, S., and Benter, I.F. (2007). Nonviral delivery of synthetic siRNAs in vivo. *The Journal of Clinical Investigation* 117(12), 3623-3632. doi: 10.1172/JCI33494.

Akuma, P., Okagu, O.D., and Udenigwe, C.C. (2019). Naturally Occurring Exosome Vesicles as Potential Delivery Vehicle for Bioactive Compounds. *Frontiers in Sustainable Food Systems* 3(23). doi: 10.3389/fsufs.2019.00023.

Alberini, C.M. (2009). Transcription factors in long-term memory and synaptic plasticity. *Physiological reviews* 89(1), 121-145. doi: 10.1152/physrev.00017.2008.

Alberini, C.M., and Kandel, E.R. (2014). The regulation of transcription in memory consolidation. *Cold Spring Harb Perspect Biol* 7(1), a021741. doi: 10.1101/cshperspect.a021741.

Albuquerque, C., Lee, C.J., Jackson, A.C., and MacDermott, A.B. (1999). Subpopulations of GABAergic and non-GABAergic rat dorsal horn neurons express Ca^{2+}-permeable AMPA receptors. *European Journal of Neuroscience* 11(8), 2758-2766.

Allison, D.W., Gelfand, V.I., Spector, I., and Craig, A.M. (1998). Role of actin in anchoring postsynaptic receptors in cultured hippocampal neurons: differential attachment of NMDA versus AMPA receptors. *J Neurosci* 18(7), 2423-2436.

Almeida, C.G., Tampellini, D., Takahashi, R.H., Greengard, P., Lin, M.T., Snyder, E.M., et al. (2005). Beta-amyloid accumulation in APP mutant neurons reduces PSD-95 and GluR1 in synapses. *Neurobiology of Disease* 20(2), 187-198. doi: https://doi.org/10.1016/j.nbd.2005.02.008.

Almkvist, O., Basun, H., Wagner, S.L., Rowe, B.A., Wahlund, L.O., and Lannfelt, L. (1997). Cerebrospinal fluid levels of alpha-secretase-cleaved soluble amyloid precursor protein mirror cognition in a Swedish family with Alzheimer disease and a gene mutation. *Arch Neurol* 54(5), 641-644.

Aloe, L., Rocco, M.L., Bianchi, P., and Manni, L. (2012). Nerve growth factor: from the early discoveries to the potential clinical use. *Journal of Translational Medicine* 10(1), 239. doi: 10.1186/1479-5876-10-239.

Alvarez-Erviti, L., Seow, Y., Yin, H., Betts, C., Lakhal, S., and Wood, M.J.A. (2011). Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nature Biotechnology* 29(4), 341-345. doi: 10.1038/nbt.1807.

Amano, T., Wada, E., Yamada, D., Zushida, K., Maeno, H., Noda, M., et al. (2008). Heightened amygdala long-term potentiation in neurotensin receptor type-1 knockout mice. *Neuropsychopharmacology* 33(13), 3135-3145. doi: 10.1038/npp.2008.38.

Amaral, D.G., Scharfman, H.E., and Lavenex, P. (2007). The dentate gyrus: fundamental neuroanatomical organization (dentate gyrus for dummies). *Prog Brain Res* 163, 3-22. doi: 10.1016/s0079-6123(07)63001-5.

An, J.J., Gharami, K., Liao, G.Y., Woo, N.H., Lau, A.G., Vanevski, F., et al. (2008). Distinct role of long 3' UTR BDNF mRNA in spine morphology and synaptic plasticity in hippocampal neurons. *Cell* 134(1), 175-187. doi: 10.1016/j.cell.2008.05.045.

Andrásfalvy, B.K., Smith, M.A., Borchardt, T., Sprengel, R., and Magee, J.C. (2003). Impaired regulation of synaptic strength in hippocampal neurons from GluR1-deficient mice. *J Physiol* 552(Pt 1), 35-45. doi: 10.1113/jphysiol.2003.045575.

Andreasson, K., and Worley, P.F. (1995). Induction of β-A activin expression by synaptic activity and during neocortical development. *Neuroscience* 69(3), 781-796. doi: https://doi.org/10.1016/0306-4522(95)00245-E.

Ang, A.L., Taguchi, T., Francis, S., Folsch, H., Murrells, L.J., Pypaert, M., et al. (2004). Recycling endosomes can serve as intermediates during transport from the Golgi to the plasma membrane of MDCK cells. *J Cell Biol* 167(3), 531-543. doi: 10.1083/jcb.200408165.

Anggono, V., and Huganir, R.L. (2012). Regulation of AMPA receptor trafficking and synaptic plasticity. *Current opinion in neurobiology* 22(3), 461-469. doi: 10.1016/j.conb.2011.12.006.

Anggono, V., Clem, R.L., and Huganir, R.L. (2011). PICK1 loss of function occludes homeostatic synaptic scaling. *J Neurosci* 31(6), 2188-2196. doi: 10.1523/jneurosci.5633-10.2011.

Angulo, M.C., Rossier, J., and Audinat, E. (1999). Postsynaptic glutamate receptors and integrative properties of fast-spiking interneurons in the rat neocortex. *J Neurophysiol* 82(3), 1295-1302. doi: 10.1152/jn.1999.82.3.1295.

Antzoulatos, E.G., Cleary, L.J., Eskin, A., Baxter, D.A., and Byrne, J.H. (2003). Desensitization of postsynaptic glutamate receptors contributes to high-frequency homosynaptic depression of aplysia sensorimotor connections. *Learn Mem* 10(5), 309-313. doi: 10.1101/lm.61403.

Aoto, J., Nam, C.I., Poon, M.M., Ting, P., and Chen, L. (2008). Synaptic signaling by all-trans retinoic acid in homeostatic synaptic plasticity. *Neuron* 60(2), 308-320. doi: 10.1016/j.neuron.2008.08.012.

Araki, W., Hattori, K., Kanemaru, K., Yokoi, Y., Omachi, Y., Takano, H., et al. (2017). Re-evaluation of soluble APP-α and APP-β in cerebrospinal fluid as potential biomarkers for early diagnosis of dementia disorders. *Biomarker research* 5, 28-28. doi: 10.1186/s40364-017-0108-5.

Araki, Y., Lin, D.-T., and Huganir, R.L. (2010). Plasma membrane insertion of the AMPA receptor GluA2 subunit is regulated by NSF binding and Q/R editing of the ion pore. *Proceedings of the National Academy of Sciences of the United States of America* 107(24), 11080-11085. doi: 10.1073/pnas.1006584107.

Aramakis, V.B., and Metherate, R. (1998). Nicotine selectively enhances NMDA receptor-mediated synaptic transmission during postnatal development in sensory neocortex. *J Neurosci* 18(20), 8485-8495. doi: 10.1523/jneurosci.18-20-08485.1998.

Aramakis, V.B., Hsieh, C.Y., Leslie, F.M., and Metherate, R. (2000). A critical period for nicotine-induced disruption of synaptic development in rat auditory cortex. *J Neurosci* 20(16), 6106-6116. doi: 10.1523/jneurosci.20-16-06106.2000.

Arancio, O., Kandel, E.R., and Hawkins, R.D. (1995). Activity-dependent long-term enhancement of transmitter release by presynaptic 3',5'-cyclic GMP in cultured hippocampal neurons. *Nature* 376(6535), 74-80. doi: 10.1038/376074a0.

Arnold, F.J.L., Hofmann, F., Bengtson, C.P., Wittmann, M., Vanhoutte, P., and Bading, H. (2005). Microelectrode array recordings of cultured hippocampal networks reveal a simple model for transcription and protein synthesis-dependent plasticity. *The Journal of physiology* 564(Pt 1), 3-19. doi: 10.1113/jphysiol.2004.077446.

Arvanov, V.L., Seebach, B.S., and Mendell, L.M. (2000). NT-3 evokes an LTP-like facilitation of AMPA/kainate receptor-mediated synaptic transmission in the neonatal rat spinal cord. *J Neurophysiol* 84(2), 752-758. doi: 10.1152/jn.2000.84.2.752.

Ashby, M.C., De La Rue, S.A., Ralph, G.S., Uney, J., Collingridge, G.L., and Henley, J.M. (2004). Removal of AMPA receptors (AMPARs) from synapses is preceded by transient endocytosis of extrasynaptic AMPARs. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 24(22), 5172-5176. doi: 10.1523/JNEUROSCI.1042-04.2004.

Ashby, M.C., Maier, S.R., Nishimune, A., and Henley, J.M. (2006). Lateral diffusion drives constitutive exchange of AMPA receptors at dendritic spines and is regulated by spine morphology. *J Neurosci* 26(26), 7046-7055. doi: 10.1523/jneurosci.1235-06.2006.

Ashley, J., Cordy, B., Lucia, D., Fradkin, L.G., Budnik, V., and Thomson, T. (2018). Retrovirus-like Gag Protein Arc1 Binds RNA and Traffics across Synaptic Boutons. *Cell* 172(1), 262-274.e211. doi: https://doi.org/10.1016/j.cell.2017.12.022.

Asrar, S., Zhou, Z., Ren, W., and Jia, Z. (2009). Ca^{2+}-permeable AMPA receptor induced long-term potentiation requires PI3/MAP kinases but not Ca/CaM-dependent kinase II. *PLoS One* 4(2), e4339. doi: 10.1371/journal.pone.0004339.

Athos, J., Impey, S., Pineda, V.V., Chen, X., and Storm, D.R. (2002). Hippocampal CRE-mediated gene expression is required for contextual memory formation. *Nat Neurosci* 5(11), 1119-1120. doi: 10.1038/nn951.

Atkins, C.M., Nozaki, N., Shigeri, Y., and Soderling, T.R. (2004). Cytoplasmic Polyadenylation Element Binding Protein-Dependent Protein Synthesis Is Regulated by Calcium/Calmodulin-Dependent

Protein Kinase II. *The Journal of Neuroscience* 24(22), 5193-5201. doi: 10.1523/jneurosci.0854-04.2004.

Aubert-Pouëssel, A., Venier-Julienne, M.C., Clavreul, A., Sergent, M., Jollivet, C., Montero-Menei, C.N., et al. (2004). In vitro study of GDNF release from biodegradable PLGA microspheres. *J Control Release* 95(3), 463-475. doi: 10.1016/j.jconrel.2003.12.012.

Autry, A.E., Adachi, M., Nosyreva, E., Na, E.S., Los, M.F., Cheng, P.F., et al. (2011). NMDA receptor blockade at rest triggers rapid behavioural antidepressant responses. *Nature* 475(7354), 91-95. doi: 10.1038/nature10130.

Aydin, D., Filippov, M.A., Tschape, J.A., Gretz, N., Prinz, M., Eils, R., et al. (2011). Comparative transcriptome profiling of amyloid precursor protein family members in the adult cortex. *BMC Genomics* 12, 160. doi: 10.1186/1471-2164-12-160.

Babu, H., Cheung, G., Kettenmann, H., Palmer, T.D., and Kempermann, G. (2007). Enriched Monolayer Precursor Cell Cultures from Micro-Dissected Adult Mouse Dentate Gyrus Yield Functional Granule Cell-Like Neurons. *PLOS ONE* 2(4), e388. doi: 10.1371/journal.pone.0000388.

Badie-Mahdavi, H., Lu, X., Behrens, M.M., and Bartfai, T. (2005). Role of galanin receptor 1 and galanin receptor 2 activation in synaptic plasticity associated with 3',5'-cyclic AMP response element-binding protein phosphorylation in the dentate gyrus: studies with a galanin receptor 2 agonist and galanin receptor 1 knockout mice. *Neuroscience* 133(2), 591-604. doi: 10.1016/j.neuroscience.2005.02.042.

Bagal, A.A., Kao, J.P., Tang, C.M., and Thompson, S.M. (2005). Long-term potentiation of exogenous glutamate responses at single dendritic spines. *Proc Natl Acad Sci U S A* 102(40), 14434-14439. doi: 10.1073/pnas.0501956102.

Bagchi, S., Fredriksson, R., and Wallén-Mackenzie, Å. (2015). In Situ Proximity Ligation Assay (PLA). *Methods Mol Biol* 1318, 149-159. doi: 10.1007/978-1-4939-2742-5_15.

Bagni, C., Mannucci, L., Dotti, C.G., and Amaldi, F. (2000). Chemical stimulation of synaptosomes modulates alpha -Ca^{2+}/calmodulin-dependent protein kinase II mRNA association to polysomes. *J Neurosci* 20(10), Rc76.

Bahrami, S., and Drabløs, F. (2016). Gene regulation in the immediate-early response process. *Advances in Biological Regulation* 62, 37-49. doi: https://doi.org/10.1016/j.jbior.2016.05.001.

Bali ZK, Nagy LV, Budai D, Hernádi I (2019) Facilitation and inhibition of firing activity and N-methyl-D-aspartate-evoked responses of CA1 hippocampal pyramidal cells by alpha7 nicotinic acetylcholine receptor selective compounds in vivo. Scientific reports 9:9324.

Bali ZK, Nagy LV, Hernádi I (2017) Alpha7 Nicotinic Acetylcholine Receptors Play a Predominant Role in the Cholinergic Potentiation of N-Methyl-D-Aspartate Evoked Firing Responses of Hippocampal CA1 Pyramidal Cells. Frontiers in Cellular Neuroscience 11:271-271.

Bancher, C., Lassmann, H., Breitschopf, H., and Jellinger, K.A. (1997). Mechanisms of cell death in Alzheimer's disease. *J Neural Transm Suppl* 50, 141-152. doi: 10.1007/978-3-7091-6842-4_14.

Banke, T.G., Bowie, D., Lee, H.-K., Huganir, R.L., Schousboe, A., and Traynelis, S.F. (2000). Control of GluR1 AMPA Receptor Function by cAMP-Dependent Protein Kinase. *The Journal of Neuroscience* 20(1), 89-102. doi: 10.1523/jneurosci.20-01-00089.2000.

Banker, G., and Goslin, K. (1998). *Culturing nerve cells.* Cambridge, Mass.: MIT Press.

Banker, G.A., and Cowan, W.M. (1977). Rat hippocampal neurons in dispersed cell culture. *Brain Res* 126(3), 397-342.

Barad, M., Bourtchouladze, R., Winder, D.G., Golan, H., and Kandel, E. (1998). Rolipram, a type IV-specific phosphodiesterase inhibitor, facilitates the establishment of long-lasting long-term potentiation and improves memory. *Proceedings of the National Academy of Sciences* 95(25), 15020-15025.

Baratta, M.V., Lamp, T., and Tallent, M.K. (2002). Somatostatin depresses long-term potentiation and Ca^{2+} signaling in mouse dentate gyrus. *J Neurophysiol* 88(6), 3078-3086. doi: 10.1152/jn.00398.2002.

Barger, S.W., and Harmon, A.D. (1997). Microglial activation by Alzheimer amyloid precursor protein and modulation by apolipoprotein E. *Nature* 388(6645), 878-881. doi: 10.1038/42257.

Barger, S.W., and Mattson, M.P. (1996a). Induction of neuroprotective kappa B-dependent transcription by secreted forms of the Alzheimer's beta-amyloid precursor. *Brain Res Mol Brain Res* 40(1), 116-126. doi: 10.1016/0169-328x(96)00036-8.

Barger, S.W., and Mattson, M.P. (1996b). Participation of gene expression in the protection against amyloid beta-peptide toxicity by the beta-amyloid precursor protein. *Ann N Y Acad Sci* 777, 303-309. doi: 10.1111/j.1749-6632.1996.tb34437.x.

Barker, A.J., Koch, S.M., Reed, J., Barres, B.A., and Ullian, E.M. (2008). Developmental control of synaptic receptivity. *J Neurosci* 28(33), 8150-8160. doi: 10.1523/jneurosci.1744-08.2008.

Barr, D.S., Lambert, N.A., Hoyt, K.L., Moore, S.D., and Wilson, W.A. (1995). Induction and reversal of long-term potentiation by low- and high-intensity theta pattern stimulation. *J Neurosci* 15(7 Pt 2), 5402-5410.

Barr, William B., and Bieliauskas, L.A. (2016). *The Oxford Handbook of History of Clinical Neuropsychology.* Oxford University Press.

Barria, A., Derkach, V., and Soderling, T. (1997a). Identification of the Ca^{2+}/calmodulin-dependent protein kinase II regulatory phosphorylation site in the alpha-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptor. *J Biol Chem* 272(52), 32727-32730. doi: 10.1074/jbc.272.52.32727.

Barria, A., Muller, D., Derkach, V., Griffith, L.C., and Soderling, T.R. (1997b). Regulatory phosphorylation of AMPA-type glutamate receptors by CaM-KII during long-term potentiation. *Science* 276(5321), 2042-2045. doi: 10.1126/science.276.5321.2042.

Barrionuevo, G., Schottler, F., and Lynch, G. (1980). The effects of repetitive low frequency stimulation on control and "potentiated" synaptic responses in the hippocampus. *Life Sciences* 27(24), 2385-2391. doi: https://doi.org/10.1016/0024-3205(80)90509-3.

Bartel, D.P. (2009). MicroRNAs: Target Recognition and Regulatory Functions. *Cell* 136(2), 215-233. doi: https://doi.org/10.1016/j.cell.2009.01.002.

Bartlett, D.W., and Davis, M.E. (2007). Effect of siRNA nuclease stability on the in vitro and in vivo kinetics of siRNA-mediated gene silencing. *Biotechnol Bioeng* 97(4), 909-921. doi: 10.1002/bit.21285.

Barykin, E.P., Mitkevich, V.A., Kozin, S.A., and Makarov, A.A. (2017). Amyloid β Modification: A Key to the Sporadic Alzheimer's Disease? *Frontiers in Genetics* 8(58). doi: 10.3389/fgene.2017.00058.

Basarsky, T.A., Parpura, V., and Haydon, P.G. (1994). Hippocampal synaptogenesis in cell culture: developmental time course of synapse formation, calcium influx, and synaptic protein distribution. *J Neurosci* 14(11 Pt 1), 6402-6411. doi: 10.1523/jneurosci.14-11-06402.1994.

Bats, C., Groc, L., and Choquet, D. (2007). The interaction between Stargazin and PSD-95 regulates AMPA receptor surface trafficking. *Neuron* 53(5), 719-734. doi: 10.1016/j.neuron.2007.01.030.

Bayer, T.A., Cappai, R., Masters, C.L., Beyreuther, K., and Multhaup, G. (1999). It all sticks together--the APP-related family of proteins and Alzheimer's disease. *Mol Psychiatry* 4(6), 524-528. doi: 10.1038/sj.mp.4000552.

Bear, M.F. (1995). Mechanism for a sliding synaptic modification threshold. *Neuron* 15(1), 1-4. doi: 10.1016/0896-6273(95)90056-x.

Bear, M.F., Cooper, L.N., and Ebner, F.F. (1987). A physiological basis for a theory of synapse modification. *Science* 237(4810), 42-48. doi: 10.1126/science.3037696.

Béïque, J.C., Na, Y., Kuhl, D., Worley, P.F., and Huganir, R.L. (2011). Arc-dependent synapse-specific homeostatic plasticity. *Proc Natl Acad Sci U S A* 108(2), 816-821. doi: 10.1073/pnas.1017914108.

Bekkers, J.M. (2011). Pyramidal neurons. *Current Biology* 21(24), R975. doi: 10.1016/j.cub.2011.10.037.

Bekkers, J.M., and Stevens, C.F. (1990). Presynaptic mechanism for long-term potentiation in the hippocampus. *Nature* 346(6286), 724-729. doi: 10.1038/346724a0.

Bell, K.F., Zheng, L., Fahrenholz, F., and Cuello, A.C. (2008). ADAM-10 over-expression increases cortical synaptogenesis. *Neurobiol Aging* 29(4), 554-565. doi: 10.1016/j.neurobiolaging.2006.11.004.

Bellone, C., and Lüscher, C. (2005). mGluRs induce a long-term depression in the ventral tegmental area that involves a switch of the subunit composition of AMPA receptors. *Eur J Neurosci* 21(5), 1280-1288. doi: 10.1111/j.1460-9568.2005.03979.x.

Bellone, C., and Lüscher, C. (2006). Cocaine triggered AMPA receptor redistribution is reversed in vivo by mGluR-dependent long-term depression. *Nature Neuroscience* 9(5), 636-641. doi: 10.1038/nn1682.

Bencsik, N., Sziber, Z., Liliom, H., Tarnok, K., Borbely, S., Gulyas, M., et al. (2015a). Protein kinase D promotes plasticity-induced F-actin stabilization in dendritic spines and regulates memory formation. *J Cell Biol* 210(5), 771-783. doi: 10.1083/jcb.201501114.

Bender CL, Sun X, Farooq M, Yang Q, Davison C, Maroteaux M, Huang Y-s, Ishikawa Y, Liu SJ (2020) Emotional Stress Induces Structural Plasticity in Bergmann Glial Cells via an AC5–CPEB3–GluA1 Pathway. The Journal of Neuroscience 40:3374-3384.

Bergström, P., Agholme, L., Nazir, F.H., Satir, T.M., Toombs, J., Wellington, H., et al. (2016a). Amyloid precursor protein expression and processing are differentially regulated during cortical neuron differentiation. *Sci Rep* 6, 29200. doi: 10.1038/srep29200.

Berlucchi, G., and Buchtel, H.A. (2009). Neuronal plasticity: historical roots and evolution of meaning. *Experimental Brain Research* 192(3), 307-319. doi: 10.1007/s00221-008-1611-6.

Bernabeu, R., and Sharp, F.R. (2000). NMDA and AMPA/Kainate Glutamate Receptors Modulate Dentate Neurogenesis and CA3 Synapsin-I in Normal and Ischemic Hippocampus. *Journal of Cerebral Blood Flow & Metabolism* 20(12), 1669-1680. doi: 10.1097/00004647-200012000-00006.

Bernabeu, R., Bevilaqua, L., Ardenghi, P., Bromberg, E., Schmitz, P., Bianchin, M., et al. (1997). Involvement of hippocampal cAMP/cAMP-dependent protein kinase signaling pathways in a late memory consolidation phase of aversively motivated learning inrats. *Proceedings of the National Academy of Sciences* 94(13), 7041-7046.

Bernardi, R., and Pandolfi, P.P. (2007). Structure, dynamics and functions of promyelocytic leukaemia nuclear bodies. *Nat Rev Mol Cell Biol* 8(12), 1006-1016. doi: 10.1038/nrm2277.

Bernhardt, R., and Matus, A. (1984). Light and electron microscopic studies of the distribution of microtubule-associated protein 2 in rat brain: a difference between dendritic and axonal cytoskeletons. *J Comp Neurol* 226(2), 203-221. doi: 10.1002/cne.902260205.

Bernier, F., Sato, Y., Matijevic, M., Desmond, H., McGrath, S., Burns, L., et al. (2013). P4–411: Clinical study of E2609, a novel BACE1 inhibitor, demonstrates target engagement and inhibition of BACE1 activity in CSF. *Alzheimer's & Dementia* 9(4S_Part_22), P886-P886. doi: 10.1016/j.jalz.2013.08.244.

Beroun, A., Nalberczak-Skóra, M., Harda, Z., Piechota, M., Ziółkowska, M., Cały, A., et al. (2018). Generation of silent synapses in dentate gyrus correlates with development of alcohol addiction. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 43(10), 1989-1999. doi: 10.1038/s41386-018-0119-4.

Berretta, N., Berton, F., Bianchi, R., Brunelli, M., Capogna, M., and Francesconi, W. (1991). Long-term Potentiation of NMDA Receptor-mediated EPSP in Guinea-pig Hippocampal Slices. *Eur J Neurosci* 3(9), 850-854. doi: 10.1111/j.1460-9568.1991.tb00096.x.

Bertram, L., Lill, C.M., and Tanzi, R.E. (2010). The Genetics of Alzheimer Disease: Back to the Future. *Neuron* 68(2), 270-281. doi: https://doi.org/10.1016/j.neuron.2010.10.013.

Bertrand, S.J., Aksenova, M.V., Aksenov, M.Y., Mactutus, C.F., and Booze, R.M. (2011). Endogenous amyloidogenesis in long-term rat hippocampal cell cultures. *BMC neuroscience* 12, 38-38. doi: 10.1186/1471-2202-12-38.

Bevan, M.J. (2010). The earliest knockouts. *Journal of immunology (Baltimore, Md. : 1950)* 184(9), 4585-4586. doi: 10.4049/jimmunol.1090023.

Bezzi, P., Carmignoto, G., Pasti, L., Vesce, S., Rossi, D., Rizzini, B.L., et al. (1998). Prostaglandins stimulate calcium-dependent glutamate release in astrocytes. *Nature* 391(6664), 281-285. doi: 10.1038/34651.

Bhattacharya S, Khatri A, Swanger SA, DiRaddo JO, Yi F, Hansen KB, Yuan H, Traynelis SF (2018) Triheteromeric GluN1/GluN2A/GluN2C NMDARs with Unique Single-Channel Properties Are the Dominant Receptor Population in Cerebellar Granule Cells. Neuron 99:315-328.e315.

Bibl, M., Gallus, M., Welge, V., Esselmann, H., Wolf, S., Rüther, E., et al. (2012). Cerebrospinal fluid amyloid-β 2-42 is decreased in Alzheimer's, but not in frontotemporal dementia. *J Neural Transm (Vienna)* 119(7), 805-813. doi: 10.1007/s00702-012-0801-3.

Bie, B., Wu, J., Foss, J.F., and Naguib, M. (2018). Amyloid fibrils induce dysfunction of hippocampal glutamatergic silent synapses. *Hippocampus* 28(8), 549-556. doi: 10.1002/hipo.22955.

Biederer, T., and Scheiffele, P. (2007). Mixed-culture assays for analyzing neuronal synapse formation. *Nature Protocols* 2(3), 670-676. doi: 10.1038/nprot.2007.92.

Bienenstock, E.L., Cooper, L.N., and Munro, P.W. (1982). Theory for the development of neuron selectivity: orientation specificity and binocular interaction in visual cortex. *J Neurosci* 2(1), 32-48.

Biou, V., Bhattacharyya, S., and Malenka, R.C. (2008). Endocytosis and recycling of AMPA receptors lacking GluR2/3. *Proceedings of the National Academy of Sciences* 105(3), 1038-1043. doi: 10.1073/pnas.0711412105.

Birnbaum, J.H., Bali, J., Rajendran, L., Nitsch, R.M., and Tackenberg, C. (2015). Calcium flux-independent NMDA receptor activity is required for Abeta oligomer-induced synaptic loss. *Cell Death Dis* 6, e1791. doi: 10.1038/cddis.2015.160.

Bissen, D., Foss, F., and Acker-Palmer, A. (2019). AMPA receptors and their minions: auxiliary proteins in AMPA receptor trafficking. *Cellular and molecular life sciences : CMLS* 76(11), 2133-2169. doi: 10.1007/s00018-019-03068-7.

Black, M.D. (2005). Therapeutic potential of positive AMPA modulators and their relationship to AMPA receptor subunits. A review of preclinical data. *Psychopharmacology (Berl)* 179(1), 154-163. doi: 10.1007/s00213-004-2065-6.

Blank, T., Nijholt, I., Eckart, K., and Spiess, J. (2002). Priming of long-term potentiation in mouse hippocampus by corticotropin-releasing factor and acute stress: implications for hippocampus-dependent learning. *J Neurosci* 22(9), 3788-3794. doi: 10.1523/jneurosci.22-09-03788.2002.

Blanpied, T.A., Scott, D.B., and Ehlers, M.D. (2002). Dynamics and regulation of clathrin coats at specialized endocytic zones of dendrites and spines. *Neuron* 36(3), 435-449. doi: 10.1016/s0896-6273(02)00979-0.

Blatow, M., Caputi, A., Burnashev, N., Monyer, H., and Rozov, A. (2003). Ca^{2+} Buffer Saturation Underlies Paired Pulse Facilitation in Calbindin-D28k-Containing Terminals. *Neuron* 38(1), 79-88. doi: https://doi.org/10.1016/S0896-6273(03)00196-X.

Bliss, T.V., and Collingridge, G.L. (1993). A synaptic model of memory: long-term potentiation in the hippocampus. *Nature* 361(6407), 31-39. doi: 10.1038/361031a0.

Bliss, T.V., and Gardner-Medwin, A.R. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the unanaestetized rabbit following stimulation of the perforant path. *J Physiol* 232(2), 357-374.

Bliss, T.V., and Lomo, T. (1973). Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. *J Physiol* 232(2), 331-356.

Bliss, T.V.P., Collingridge, G.L., and Morris, R.G.M. (2013). Synaptic plasticity in health and disease: introduction and overview. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 369(1633), 20130129-20130129. doi: 10.1098/rstb.2013.0129.

Bliss, T.V.P., Collingridge, G.L., Morris, R.G.M., Bortolotto, Z.A., Lauri, S., Isaac, J.T.R., et al. (2003). Kainate receptors and the induction of mossy fibre long-term potentiation. *Philosophical*

Transactions of the Royal Society of London. Series B: Biological Sciences 358(1432), 657-666. doi: doi:10.1098/rstb.2002.1216.

Blitzer, R.D., Wong, T., Nouranifar, R., Iyengar, R., and Landau, E.M. (1995). Postsynaptic cAMP pathway gates early LTP in hippocampal CA1 region. *Neuron* 15(6), 1403-1414. doi: 10.1016/0896-6273(95)90018-7.

Bloomer, W.A., VanDongen, H.M., and VanDongen, A.M. (2008). Arc/Arg3.1 translation is controlled by convergent N-methyl-D-aspartate and Gs-coupled receptor signaling pathways. *J Biol Chem* 283(1), 582-592. doi: 10.1074/jbc.M702451200.

Blundon, J.A., and Zakharenko, S.S. (2008). Dissecting the components of long-term potentiation. *The Neuroscientist : a review journal bringing neurobiology, neurology and psychiatry* 14(6), 598-608. doi: 10.1177/1073858408320643.

Bocchio, M., Nabavi, S., and Capogna, M. (2017). Synaptic Plasticity, Engrams, and Network Oscillations in Amygdala Circuits for Storage and Retrieval of Emotional Memories. *Neuron* 94(4), 731-743. doi: https://doi.org/10.1016/j.neuron.2017.03.022.

Boehm, J., Kang, M.-G., Johnson, R.C., Esteban, J., Huganir, R.L., and Malinow, R. (2006). Synaptic Incorporation of AMPA Receptors during LTP Is Controlled by a PKC Phosphorylation Site on GluR1. *Neuron* 51(2), 213-225. doi: https://doi.org/10.1016/j.neuron.2006.06.013.

Bollen, E., Puzzo, D., Rutten, K., Privitera, L., De Vry, J., Vanmierlo, T., et al. (2014). Improved Long-Term Memory via Enhancing cGMP-PKG Signaling Requires cAMP-PKA Signaling. *Neuropsychopharmacology* 39(11), 2497-2505. doi: 10.1038/npp.2014.106.

Bolshakov, V.Y., Golan, H., Kandel, E.R., and Siegelbaum, S.A. (1997). Recruitment of New Sites of Synaptic Transmission During the cAMP-Dependent Late Phase of LTP at CA3–CA1 Synapses in the Hippocampus. *Neuron* 19(3), 635-651. doi: https://doi.org/10.1016/S0896-6273(00)80377-3.

Bonini, J.S., Da Silva, W.C., Bevilaqua, L.R.M., Medina, J.H., Izquierdo, I., and Cammarota, M. (2007). On the participation of hippocampal PKC in acquisition, consolidation and reconsolidation of spatial memory. *Neuroscience* 147(1), 37-45. doi: https://doi.org/10.1016/j.neuroscience.2007.04.013.

Boos, D.D., Stefanski, L.A. (2013). *Essential Statistical Inference: Theory and Methods.* New York: Springer.

Borgdorff, A.J., and Choquet, D. (2002). Regulation of AMPA receptor lateral movements. *Nature* 417(6889), 649-653. doi: 10.1038/nature00780.

Borst, J.G.G., and Sakmann, B. (1998). Facilitation of presynaptic calcium currents in the rat brainstem. *The Journal of Physiology* 513(Pt 1), 149-155. doi: 10.1111/j.1469-7793.1998.149by.x.

Bortolotto, Z.A., Fitzjohn, S.M., and Collingridge, G.L. (1999). Roles of metabotropic glutamate receptors in LTP and LTD in. *Current Opinion in Neurobiology* 9(3), 299-304. doi: https://doi.org/10.1016/S0959-4388(99)80044-0.

Bosch, M., Castro, J., Saneyoshi, T., Matsuno, H., Sur, M., and Hayashi, Y. (2014). Structural and molecular remodeling of dendritic spine substructures during long-term potentiation. *Neuron* 82(2), 444-459. doi: 10.1016/j.neuron.2014.03.021.

Boudreau, A.C., and Wolf, M.E. (2005). Behavioral sensitization to cocaine is associated with increased AMPA receptor surface expression in the nucleus accumbens. *J Neurosci* 25(40), 9144-9151. doi: 10.1523/jneurosci.2252-05.2005.

Bourtchouladze, R., Abel, T., Berman, N., Gordon, R., Lapidus, K., and Kandel, E.R. (1998). Different training procedures recruit either one or two critical periods for contextual memory consolidation, each of which requires protein synthesis and PKA. *Learn Mem* 5(4-5), 365-374.

Bowen, A.B., Bourke, A.M., Hiester, B.G., Hanus, C., and Kennedy, M.J. (2017). Golgi-independent secretory trafficking through recycling endosomes in neuronal dendrites and spines. *eLife* 6, e27362. doi: 10.7554/eLife.27362.

Bowling, H., Bhattacharya, A., Zhang, G., Lebowitz, J.Z., Alam, D., Smith, P.T., et al. (2016). BONLAC: A combinatorial proteomic technique to measure stimulus-induced translational profiles in brain slices. Neuropharmacology 100, 76-89. doi: 10.1016/j.neuropharm.2015.07.017.

Bramham, C.R., Alme, M.N., Bittins, M., Kuipers, S.D., Nair, R.R., Pai, B., et al. (2010). The Arc of synaptic memory. *Experimental brain research* 200(2), 125-140. doi: 10.1007/s00221-009-1959-2.

Bramham, C.R., and Sarvey, J.M. (1996). Endogenous Activation of μ and δ-1 Opioid Receptors Is Required for Long-Term Potentiation Induction in the Lateral Perforant Path: Dependence on GABAergic Inhibition. *The Journal of Neuroscience* 16(24), 8123-8131. doi: 10.1523/jneurosci.16-24-08123.1996.

Bramham, C.R., Worley, P.F., Moore, M.J., and Guzowski, J.F. (2008). The Immediate Early Gene Arc/Arg3.1: Regulation, Mechanisms, and Function. *The Journal of Neuroscience* 28(46), 11760-11767. doi: 10.1523/jneurosci.3864-08.2008.

Breese, C.R., Logel, J., Adams, C., and Leonard, S.S. (1996). Regional gene expression of the glutamate receptor subtypes GluR1, GluR2, and GluR3 in human postmortem brain. *J Mol Neurosci* 7(4), 277-289. doi: 10.1007/bf02737065.

Bregman, L. (1899). Ueber den "Automatisme ambulatoire"("Fugues","Dromomania")[On the "automatisme ambulatoire"("fugues","dromomania")]. *Neurologisches Centralblatt* 18, 776-781.

Brenman, J.E., Chao, D.S., Gee, S.H., McGee, A.W., Craven, S.E., Santillano, D.R., et al. (1996). Interaction of nitric oxide synthase with the postsynaptic density protein PSD-95 and alpha1-syntrophin mediated by PDZ domains. *Cell* 84(5), 757-767. doi: 10.1016/s0092-8674(00)81053-3.

Bretin, S., Louis, C., Seguin, L., Wagner, S., Thomas, J.-Y., Challal, S., et al. (2017). Pharmacological characterisation of S 47445, a novel positive allosteric modulator of AMPA receptors. *PLOS ONE* 12(9), e0184429. doi: 10.1371/journal.pone.0184429.

Brewer, G.J. (1997). Isolation and culture of adult rat hippocampal neurons. *Journal of Neuroscience Methods* 71(2), 143-155. doi: https://doi.org/10.1016/S0165-0270(96)00136-7.

Brewer, G.J., and Torricelli, J.R. (2007). Isolation and culture of adult neurons and neurospheres. *Nature Protocols* 2(6), 1490-1498. doi: 10.1038/nprot.2007.207.

Brewer, G.J., Torricelli, J.R., Evege, E.K., and Price, P.J. (1993). Optimized survival of hippocampal neurons in B27-supplemented Neurobasal, a new serum-free medium combination. *J Neurosci Res* 35(5), 567-576. doi: 10.1002/jnr.490350513.

Brinkmalm, G., Brinkmalm, A., Bourgeois, P., Persson, R., Hansson, O., Portelius, E., et al. (2013). Soluble amyloid precursor protein α and β in CSF in Alzheimer's disease. *Brain Res* 1513, 117-126. doi: 10.1016/j.brainres.2013.03.019.

Brodmann, K. (1909). *Vergleichende Lokalisationslehre der Grosshirnrinde in ihren Prinzipien dargestellt auf Grund des Zellenbaues.* Barth.

Brown, T.E., Lee, B.R., Mu, P., Ferguson, D., Dietz, D., Ohnishi, Y.N., et al. (2011). A silent synapse-based mechanism for cocaine-induced locomotor sensitization. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 31(22), 8163-8174. doi: 10.1523/JNEUROSCI.0016-11.2011.

Browning, D.D., McShane, M.P., Marty, C., and Ye, R.D. (2000). Nitric oxide activation of p38 mitogen-activated protein kinase in 293T fibroblasts requires cGMP-dependent protein kinase. *J Biol Chem* 275(4), 2811-2816.

Buard, I., Coultrap, S.J., Freund, R.K., Lee, Y.S., Dell'Acqua, M.L., Silva, A.J., et al. (2010). CaMKII "autonomy" is required for initiating but not for maintaining neuronal long-term information storage. *J Neurosci* 30(24), 8214-8220. doi: 10.1523/jneurosci.1469-10.2010.

Burgoyne, R.D., and Weiss, J.L. (2001). The neuronal calcium sensor family of Ca^{2+}-binding proteins. *Biochemical Journal* 353(Pt 1), 1-12.

Burrone, J., O'Byrne, M., and Murthy, V.N. (2002). Multiple forms of synaptic plasticity triggered by selective suppression of activity in individual neurons. *Nature* 420(6914), 414-418. doi: 10.1038/nature01242.

Burwell, R.D., and Amaral, D.G. (1998). Cortical afferents of the perirhinal, postrhinal, and entorhinal cortices of the rat. *J Comp Neurol* 398(2), 179-205. doi: 10.1002/(sici)1096-9861(19980824)398:2<179::aid-cne3>3.0.co;2-y.

Busetto, G., Higley, M.J., and Sabatini, B.L. (2008). Developmental presence and disappearance of postsynaptically silent synapses on dendritic spines of rat layer 2/3 pyramidal neurons. *The Journal of Physiology* 586(6), 1519-1527. doi: 10.1113/jphysiol.2007.149336.

Byrne, J.H. (2010). *Concise Learning and Memory: The Editor's Selection.* Elsevier Science.

Caceres, A., Binder, L.I., Payne, M.R., Bender, P., Rebhun, L., and Steward, O. (1984). Differential subcellular localization of tubulin and the microtubule-associated protein MAP2 in brain tissue as revealed by immunocytochemistry with monoclonal hybridoma antibodies. *J Neurosci* 4(2), 394-410.

Caillard, O., Moreno, H., Schwaller, B., Llano, I., Celio, M.R., and Marty, A. (2000). Role of the calcium-binding protein parvalbumin in short-term synaptic plasticity. *Proceedings of the National Academy of Sciences* 97(24), 13372-13377. doi: 10.1073/pnas.230362997.

Cajal, S.R. (1893). La retine des vertebres. *Cellule* 9, 119-255.

Cajal, S.R. (1896). Les épines collatérales das cellules du cerveau colorées au bleu de méthylène. *Rev. Trim. Microgr. 1, 5–19.*

Cajigas, I.J., Tushev, G., Will, T.J., tom Dieck, S., Fuerst, N., and Schuman, E.M. (2012). The local transcriptome in the synaptic neuropil revealed by deep sequencing and high-resolution imaging. *Neuron* 74(3), 453-466. doi: 10.1016/j.neuron.2012.02.036.

Cajigas, I.J., Will, T., and Schuman, E.M. (2010). Protein homeostasis and synaptic plasticity. *Embo j* 29(16), 2746-2752. doi: 10.1038/emboj.2010.173.

Calabrese, B., Saffin, J.-M., and Halpain, S. (2014). Activity-dependent dendritic spine shrinkage and growth involve downregulation of cofilin via distinct mechanisms. *PloS one* 9(4), e94787-e94787. doi: 10.1371/journal.pone.0094787.

Caldeira, M.V., Melo, C.V., Pereira, D.B., Carvalho, R., Correia, S.S., Backos, D.S., et al. (2007). Brain-derived Neurotrophic Factor Regulates the Expression and Synaptic Delivery of α-Amino-3-hydroxy-5-methyl-4-isoxazole Propionic Acid Receptor Subunits in Hippocampal Neurons. *Journal of Biological Chemistry* 282(17), 12619-12628. doi: 10.1074/jbc.M700607200.

Camina, E., and Güell, F. (2017). The Neuroanatomical, Neurophysiological and Psychological Basis of Memory: Current Models and Their Origins. *Frontiers in Pharmacology* 8(438). doi: 10.3389/fphar.2017.00438.

Cammarota, M., Bevilaqua, L.R., Ardenghi, P., Paratcha, G., Levi de Stein, M., Izquierdo, I., et al. (2000). Learning-associated activation of nuclear MAPK, CREB and Elk-1, along with Fos production, in the rat hippocampus after a one-trial avoidance learning: abolition by NMDA receptor blockade. *Brain Res Mol Brain Res* 76(1), 36-46.

Camp, A.J. (2012). Intrinsic neuronal excitability: a role in homeostasis and disease. *Frontiers in neurology* 3, 50-50. doi: 10.3389/fneur.2012.00050.

Cao, G., and Harris, K.M. (2014). Augmenting saturated LTP by broadly spaced episodes of theta-burst stimulation in hippocampal area CA1 of adult rats and mice. *Journal of Neurophysiology* 112(8), 1916-1924. doi: 10.1152/jn.00297.2014.

Cao, Q., Huang, Y.-S., Kan, M.-C., and Richter, J.D. (2005). Amyloid Precursor Proteins Anchor CPEB to Membranes and Promote Polyadenylation-Induced Translation. *Molecular and Cellular Biology* 25(24), 10930-10939. doi: 10.1128/mcb.25.24.10930-10939.2005.

Carino, C., Fibuch, E.E., Mao, L.-M., and Wang, J.Q. (2012). Dynamic loss of surface-expressed AMPA receptors in mouse cortical and striatal neurons during anesthesia. *Journal of neuroscience research* 90(1), 315-323. doi: 10.1002/jnr.22749.

Casimiro, T.M., Nawy, S., and Carroll, R.C. (2013). Molecular mechanisms underlying activity-dependent AMPA receptor cycling in retinal ganglion cells. *Mol Cell Neurosci* 56, 384-392. doi: 10.1016/j.mcn.2013.07.010.

Catlin, R., Taylor, A., Ditchek, L., Burnett, S., Khan, S., Todd, O., et al. (2016). Using Cultured Mammalian Neurons to Study Cellular Processes and Neurodegeneration: A Suite of Undergraduate Lab Exercises. *Journal of undergraduate neuroscience education : JUNE : a publication of FUN, Faculty for Undergraduate Neuroscience* 14(2), A132-A137.

Catterall, W.A., and Few, A.P. (2008). Calcium channel regulation and presynaptic plasticity. *Neuron* 59(6), 882-901. doi: 10.1016/j.neuron.2008.09.005.

Chafai, M., Corbani, M., Guillon, G., and Desarménien, M.G. (2012). Vasopressin Inhibits LTP in the CA2 Mouse Hippocampal Area. *PLOS ONE* 7(12), e49708. doi: 10.1371/journal.pone.0049708.

Chalimoniuk, M., Głód, B., and Strosznajder, J. (1996). NMDA receptor mediated nitric oxide dependent cGMP synthesis in brain cortex and hippocampus. Effect of ischemia on NO related biochemical processes during reperfusion. *Neurol Neurochir Pol* 30 Suppl 2, 65-84.

Chamberland, S., and Topolnik, L. (2012). Inhibitory control of hippocampal inhibitory neurons. *Frontiers in Neuroscience* 6(165). doi: 10.3389/fnins.2012.00165.

Chang, E.H., Savage, M.J., Flood, D.G., Thomas, J.M., Levy, R.B., Mahadomrongkul, V., et al. (2006). AMPA receptor downscaling at the onset of Alzheimer's disease pathology in double knockin mice. *Proceedings of the National Academy of Sciences of the United States of America* 103(9), 3410-3415. doi: 10.1073/pnas.0507313103.

Chappell, A.S., and Witte, M.M. (2004). "AMPA Potentiation as a Treatment Option for Alzheimer's Disease," in *Alzheimer's Disease: A Physician's Guide to Practical Management,* eds. R.W. Richter & B.Z. Richter. (Totowa, NJ: Humana Press), 309-314.

Charras, G.T. (2008). A short history of blebbing. *Journal of Microscopy* 231(3), 466-478. doi: doi:10.1111/j.1365-2818.2008.02059.x.

Chartier-Harlin, M.C., Dachsel, J.C., Vilarino-Guell, C., Lincoln, S.J., Lepretre, F., Hulihan, M.M., et al. (2011). Translation initiator EIF4G1 mutations in familial Parkinson disease. *Am J Hum Genet* 89(3), 398-406. doi: 10.1016/j.ajhg.2011.08.009.

Chasseigneaux, S., Dinc, L., Rose, C., Chabret, C., Coulpier, F., Topilko, P., et al. (2011). Secreted amyloid precursor protein β and secreted amyloid precursor protein α induce axon outgrowth in vitro through Egr1 signaling pathway. *PLoS One* 6(1), e16301. doi: 10.1371/journal.pone.0016301.

Chawla, M.K., Gray, D.T., Nguyen, C., Dhaliwal, H., Zempare, M., Okuno, H., et al. (2018a). Seizure-Induced Arc mRNA Expression Thresholds in Rat Hippocampus and Perirhinal Cortex. *Frontiers in Systems Neuroscience* 12(53). doi: 10.3389/fnsys.2018.00053.

Chawla, M.K., Sutherland, V.L., Olson, K., McNaughton, B.L., and Barnes, C.A. (2018b). Behavior-driven arc expression is reduced in all ventral hippocampal subfields compared to CA1, CA3, and dentate gyrus in rat dorsal hippocampus. *Hippocampus* 28(2), 178-185. doi: 10.1002/hipo.22820.

Chen, C., Hu, Q., Yan, J., Yang, X., Shi, X., Lei, J., et al. (2009). Early inhibition of HIF-1alpha with small interfering RNA reduces ischemic-reperfused brain injury in rats. *Neurobiol Dis* 33(3), 509-517. doi: 10.1016/j.nbd.2008.12.010.

Chen, H.-X., Jiang, M., Akakin, D., and Roper, S.N. (2009). Long-term potentiation of excitatory synapses on neocortical somatostatin-expressing interneurons. *Journal of neurophysiology* 102(6), 3251-3259. doi: 10.1152/jn.00641.2009.

Chen, L.Y., Rex, C.S., Casale, M.S., Gall, C.M., and Lynch, G. (2007). Changes in synaptic morphology accompany actin signaling during LTP. *J Neurosci* 27(20), 5363-5372. doi: 10.1523/jneurosci.0164-07.2007.

Chen, T., Zhu, J., Yang, L.-K., Feng, Y., Lin, W., and Wang, Y.-H. (2017). Glutamate-induced rapid induction of Arc/Arg3.1 requires NMDA receptor-mediated phosphorylation of ERK and CREB. *Neuroscience Letters* 661, 23-28. doi: https://doi.org/10.1016/j.neulet.2017.09.024.

Chen, W., Feng, Y., Chen, D., and Wandinger-Ness, A. (1998). Rab11 is required for trans-golgi network-to-plasma membrane transport and a preferential target for GDP dissociation inhibitor. *Mol Biol Cell* 9(11), 3241-3257.

Chen, X., Levy, J.M., Hou, A., Winters, C., Azzam, R., Sousa, A.A., et al. (2015). PSD-95 family MAGUKs are essential for anchoring AMPA and NMDA receptor complexes at the postsynaptic density. *Proceedings of the National Academy of Sciences of the United States of America* 112(50), E6983-E6992. doi: 10.1073/pnas.1517045112.

Chen, X., Li, X., Wong, Y.T., Zheng, X., Wang, H., Peng, Y., et al. (2019). Cholecystokinin release triggered by NMDA receptors produces LTP and sound–sound associative memory. *Proceedings of the National Academy of Sciences* 116(13), 6397-6406. doi: 10.1073/pnas.1816833116.

Chen, X.H., Siman, R., Iwata, A., Meaney, D.F., Trojanowski, J.Q., and Smith, D.H. (2004). Long-term accumulation of amyloid-beta, beta-secretase, presenilin-1, and caspase-3 in damaged axons following brain trauma. *Am J Pathol* 165(2), 357-371.

Chen, Y., Zhuang, S., Cassenaer, S., Casteel, D.E., Gudi, T., Boss, G.R., et al. (2003). Synergism between calcium and cyclic GMP in cyclic AMP response element-dependent transcriptional regulation requires cooperation between CREB and C/EBP-beta. *Mol Cell Biol* 23(12), 4066-4082. doi: 10.1128/mcb.23.12.4066-4082.2003.

Cheng, R., Zhang, F., Li, M., Wo, X., Su, Y.-W., and Wang, W. (2019). Influence of Fixation and Permeabilization on the Mass Density of Single Cells: A Surface Plasmon Resonance Imaging Study. *Frontiers in Chemistry* 7(588). doi: 10.3389/fchem.2019.00588.

Chéreau, R., Saraceno, G.E., Angibaud, J., Cattaert, D., and Nägerl, U.V. (2017). Superresolution imaging reveals activity-dependent plasticity of axon morphology linked to changes in action potential conduction velocity. *Proceedings of the National Academy of Sciences* 114(6), 1401-1406. doi: 10.1073/pnas.1607541114.

Chirila, A.M., Brown, T.E., Bishop, R.A., Bellono, N.W., Pucci, F.G., and Kauer, J.A. (2014). Long-term potentiation of glycinergic synapses triggered by interleukin 1β. *Proceedings of the National Academy of Sciences of the United States of America* 111(22), 8263-8268. doi: 10.1073/pnas.1401013111.

Cho, S., Wood, A., and Bowlby, M.R. (2007). Brain slices as models for neurodegenerative disease and screening platforms to identify novel therapeutics. *Curr Neuropharmacol* 5(1), 19-33.

Choquet, D., and Triller, A. (2003). The role of receptor diffusion in the organization of the postsynaptic membrane. *Nat Rev Neurosci* 4(4), 251-265.

Chotiner, J.K., Nielson, J., Farris, S., Lewandowski, G., Huang, F., Banos, K., et al. (2010). Assessment of the role of MAP kinase in mediating activity-dependent transcriptional activation of the immediate early gene Arc/Arg3.1 in the dentate gyrus in vivo. *Learn Mem* 17(2), 117-129. doi: 10.1101/lm.1585910.

Chow, V.W., Mattson, M.P., Wong, P.C., and Gleichmann, M. (2010). An Overview of APP Processing Enzymes and Products. *Neuromolecular Med* 12(1), 1-12. doi: 10.1007/s12017-009-8104-z.

Chowdhury, S., Shepherd, J.D., Okuno, H., Lyford, G., Petralia, R.S., Plath, N., et al. (2006). Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking. *Neuron* 52(3), 445-459. doi: 10.1016/j.neuron.2006.08.033.

Christie, B.R., and Abraham, W.C. (1992). Priming of associative long-term depression in the dentate gyrus by theta frequency synaptic activity. *Neuron* 9(1), 79-84.

Christopherson, K.S., Hillier, B.J., Lim, W.A., and Bredt, D.S. (1999). PSD-95 Assembles a Ternary Complex with theN-Methyl-d-aspartic Acid Receptor and a Bivalent Neuronal NO Synthase PDZ Domain. *Journal of Biological Chemistry* 274(39), 27467-27473. doi: 10.1074/jbc.274.39.27467.

Chung, H.J., Xia, J., Scannevin, R.H., Zhang, X., and Huganir, R.L. (2000). Phosphorylation of the AMPA receptor subunit GluR2 differentially regulates its interaction with PDZ domain-containing proteins. *J Neurosci* 20(19), 7258-7267. doi: 10.1523/jneurosci.20-19-07258.2000.

Churchill, L., Swanson, C.J., Urbina, M., and Kalivas, P.W. (1999). Repeated cocaine alters glutamate receptor subunit levels in the nucleus accumbens and ventral tegmental area of rats that develop behavioral sensitization. *J Neurochem* 72(6), 2397-2403. doi: 10.1046/j.1471-4159.1999.0722397.x.

Cingolani, L.A., and Goda, Y. (2008). Actin in action: the interplay between the actin cytoskeleton and synaptic efficacy. *Nat Rev Neurosci* 9(5), 344-356.
Cirrito, J.R., May, P.C., O'Dell, M.A., Taylor, J.W., Parsadanian, M., Cramer, J.W., et al. (2003). In vivo assessment of brain interstitial fluid with microdialysis reveals plaque-associated changes in amyloid-beta metabolism and half-life. *J Neurosci* 23(26), 8844-8853.
Citri, A., and Malenka, R.C. (2008). Synaptic Plasticity: Multiple Forms, Functions, and Mechanisms. *Neuropsychopharmacology* 33(1), 18-41. doi: 10.1038/sj.npp.1301559.
Citri, A., Bhattacharyya, S., Ma, C., Morishita, W., Fang, S., Rizo, J., et al. (2010). Calcium binding to PICK1 is essential for the intracellular retention of AMPA receptors underlying long-term depression. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 30(49), 16437-16452. doi: 10.1523/JNEUROSCI.4478-10.2010.
Claasen, A.M., Guevremont, D., Mason-Parker, S.E., Bourne, K., Tate, W.P., Abraham, W.C., et al. (2009). Secreted amyloid precursor protein-alpha upregulates synaptic protein synthesis by a protein kinase G-dependent mechanism. *Neurosci Lett* 460(1), 92-96. doi: 10.1016/j.neulet.2009.05.040.
Clarris, H.J., Nurcombe, V., Small, D.H., Beyreuther, K., and Masters, C.L. (1994). Secretion of nerve growth factor from septum stimulates neurite outgrowth and release of the amyloid protein precursor of Alzheimer's disease from hippocampal explants. *J Neurosci Res* 38(3), 248-258. doi: 10.1002/jnr.490380303.
Clem, R.L., and Barth, A. (2006). Pathway-Specific Trafficking of Native AMPARs by In Vivo Experience. *Neuron* 49(5), 663-670. doi: https://doi.org/10.1016/j.neuron.2006.01.019.
Clem, R.L., and Huganir, R.L. (2010). Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. *Science* 330(6007), 1108-1112. doi: 10.1126/science.1195298.
Clem, R.L., and Huganir, R.L. (2013). Norepinephrine Enhances a Discrete Form of Long-Term Depression during Fear Memory Storage. *The Journal of Neuroscience* 33(29), 11825-11832. doi: 10.1523/jneurosci.3317-12.2013.
Clem, R.L., Celikel, T., and Barth, A.L. (2008). Ongoing in vivo experience triggers synaptic metaplasticity in the neocortex. *Science* 319(5859), 101-104. doi: 10.1126/science.1143808.
Coelho, F.G., Vital, T.M., Stein, A.M., Arantes, F.J., Rueda, A.V., Camarini, R., et al. (2014). Acute aerobic exercise increases brain-derived neurotrophic factor levels in elderly with Alzheimer's disease. *J Alzheimers Dis* 39(2), 401-408. doi: 10.3233/jad-131073.
Cognet, L., Groc, L., Lounis, B., and Choquet, D. (2006). Multiple routes for glutamate receptor trafficking: surface diffusion and membrane traffic cooperate to bring receptors to synapses. *Sci STKE* 2006(327), pe13. doi: 10.1126/stke.3272006pe13.
Colciaghi, F., Borroni, B., Pastorino, L., Marcello, E., Zimmermann, M., Cattabeni, F., et al. (2002). [alpha]-Secretase ADAM10 as well as [alpha]APPs is reduced in platelets and CSF of Alzheimer disease patients. *Mol Med* 8(2), 67-74.
Colledge, M., Dean, R.A., Scott, G.K., Langeberg, L.K., Huganir, R.L., and Scott, J.D. (2000). Targeting of PKA to glutamate receptors through a MAGUK-AKAP complex. *Neuron* 27(1), 107-119. doi: 10.1016/s0896-6273(00)00013-1.
Colley, P.A., Sheu, F.S., and Routtenberg, A. (1990). Inhibition of protein kinase C blocks two components of LTP persistence, leaving initial potentiation intact. *J Neurosci* 10(10), 3353-3360.
Collin, C., Miyaguchi, K., and Segal, M. (1997). Dendritic spine density and LTP induction in cultured hippocampal slices. *J Neurophysiol* 77(3), 1614-1623. doi: 10.1152/jn.1997.77.3.1614.
Collingridge, G.L., Isaac, J.T.R., and Wang, Y.T. (2004). Receptor trafficking and synaptic plasticity. *Nat Rev Neurosci* 5(12), 952-962.
Collingridge, G.L., Kehl, S.J., and McLennan, H. (1983). Excitatory amino acids in synaptic transmission in the Schaffer collateral-commissural pathway of the rat hippocampus. *The Journal of physiology* 334, 33-46. doi: 10.1113/jphysiol.1983.sp014478.

Conde Guerri, B., Sinues Porta, E., Arrazola Schlamilch, M., Comunas Gonzalez, F., and Calatayud Maldonado, V. (1989a). Effects of glia-conditioned medium on primary cultures of central neurons. *Histol Histopathol* 4(2), 217-222.

Conde Guerri, B., Sinués Porta, E., Arrazola Schlamilch, M., Comuñas González, F., and Calatayud Maldonado, V. (1989b). Effects of glia-conditioned medium on primary cultures of central neurons. *Histol Histopathol* 4(2), 217-222.

Conner, J.M., Franks, K.M., Titterness, A.K., Russell, K., Merrill, D.A., Christie, B.R., et al. (2009). NGF Is Essential for Hippocampal Plasticity and Learning. *The Journal of Neuroscience* 29(35), 10883-10889. doi: 10.1523/jneurosci.2594-09.2009.

Connor, J.R., and Diamond, M.C. (1982). A comparison of dendritic spine number and type on pyramidal neurons of the visual cortex of old adult rats from social or isolated environments. *Journal of Comparative Neurology* 210(1), 99-106.

Conrad, K.L., Tseng, K.Y., Uejima, J.L., Reimers, J.M., Heng, L.J., Shaham, Y., et al. (2008). Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature* 454(7200), 118-121. doi: 10.1038/nature06995.

Contestabile, A. (2000). Roles of NMDA receptor activity and nitric oxide production in brain development. *Brain Res Brain Res Rev* 32(2-3), 476-509. doi: 10.1016/s0165-0173(00)00018-7.

Coombs, I.D., MacLean, D.M., Jayaraman, V., Farrant, M., and Cull-Candy, S.G. (2017). Dual Effects of TARP γ-2 on Glutamate Efficacy Can Account for AMPA Receptor Autoinactivation. *Cell reports* 20(5), 1123-1135. doi: 10.1016/j.celrep.2017.07.014.

Cooney, J.R., Hurlburt, J.L., Selig, D.K., Harris, K.M., and Fiala, J.C. (2002). Endosomal compartments serve multiple hippocampal dendritic spines from a widespread rather than a local store of recycling membrane. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 22(6), 2215-2224. doi: 10.1523/JNEUROSCI.22-06-02215.2002.

Copanaki, E., Chang, S., Vlachos, A., Tschape, J.A., Muller, U.C., Kogel, D., et al. (2010). sAPPalpha antagonizes dendritic degeneration and neuron death triggered by proteasomal stress. *Mol Cell Neurosci* 44(4), 386-393. doi: 10.1016/j.mcn.2010.04.007.

Correia, S.S., Bassani, S., Brown, T.C., Lisé, M.F., Backos, D.S., El-Husseini, A., et al. (2008). Motor protein-dependent transport of AMPA receptors into spines during long-term potentiation. *Nat Neurosci* 11(4), 457-466. doi: 10.1038/nn2063.

Corrigan, F., Thornton, E., Roisman, L.C., Leonard, A.V., Vink, R., Blumbergs, P.C., et al. (2014). The neuroprotective activity of the amyloid precursor protein against traumatic brain injury is mediated via the heparin binding site in residues 96-110. *J Neurochem* 128(1), 196-204. doi: 10.1111/jnc.12391.

Corrigan, F., Vink, R., Blumbergs, P.C., Masters, C.L., Cappai, R., and van den Heuvel, C. (2012). sAPPalpha rescues deficits in amyloid precursor protein knockout mice following focal traumatic brain injury. *J Neurochem* 122(1), 208-220. doi: 10.1111/j.1471-4159.2012.07761.x.

Cortese, G.P., Zhu, M., Williams, D., Heath, S., and Waites, C.L. (2016). Parkin Deficiency Reduces Hippocampal Glutamatergic Neurotransmission by Impairing AMPA Receptor Endocytosis. *J Neurosci* 36(48), 12243-12258. doi: 10.1523/jneurosci.1473-16.2016.

Corti, C., Xuereb, J.H., Crepaldi, L., Corsi, M., Michielin, F., and Ferraguti, F. (2011). Altered levels of glutamatergic receptors and Na+/K+ ATPase-alpha1 in the prefrontal cortex of subjects with schizophrenia. *Schizophr Res* 128(1-3), 7-14. doi: 10.1016/j.schres.2011.01.021.

Costa, M.C.R., Mani, F., Santoro, W., Espreafico, E.M., and Larson, R.E. (1999). Brain myosin-V, a calmodulin-carrying myosin, binds to calmodulin-dependent protein kinase II and activates its kinase activity. *Journal of Biological Chemistry* 274(22), 15811-15819.

Cottrell, J.R., Borok, E., Horvath, T.L., and Nedivi, E. (2004). CPG2: a brain- and synapse-specific protein that regulates the endocytosis of glutamate receptors. *Neuron* 44(4), 677-690. doi: 10.1016/j.neuron.2004.10.025.

Cousins, S.L., Hoey, S.E., Anne Stephenson, F., and Perkinton, M.S. (2009). Amyloid precursor protein 695 associates with assembled NR2A- and NR2B-containing NMDA receptors to result in the

enhancement of their cell surface delivery. *J Neurochem* 111(6), 1501-1513. doi: 10.1111/j.1471-4159.2009.06424.x.

Coussens, L., Parker, P., Rhee, L., Yang-Feng, T., Chen, E., Waterfield, M., et al. (1986). Multiple, distinct forms of bovine and human protein kinase C suggest diversity in cellular signaling pathways. *Science* 233(4766), 859-866. doi: 10.1126/science.3755548.

Cracco, J.B., Serrano, P., Moskowitz, S.I., Bergold, P.J., and Sacktor, T.C. (2005). Protein synthesis-dependent LTP in isolated dendrites of CA1 pyramidal cells. *Hippocampus* 15(5), 551-556. doi: 10.1002/hipo.20078.

Craig, T.J., Jaafari, N., Petrovic, M.M., Jacobs, S.C., Rubin, P.P., Mellor, J.R., et al. (2012). Homeostatic synaptic scaling is regulated by protein SUMOylation. *The Journal of biological chemistry* 287(27), 22781-22788. doi: 10.1074/jbc.M112.356337.

Crick, F. (1984). Memory and molecular turnover. *Nature* 312(5990), 101.

Cunha-Reis, D., Aidil-Carvalho Mde, F., and Ribeiro, J.A. (2014). Endogenous inhibition of hippocampal LTD and depotentiation by vasoactive intestinal peptide VPAC1 receptors. *Hippocampus* 24(11), 1353-1363. doi: 10.1002/hipo.22316.

Cunha, C., Brambilla, R., and Thomas, K. (2010). A simple role for BDNF in learning and memory? *Frontiers in Molecular Neuroscience* 3(1). doi: 10.3389/neuro.02.001.2010.

Czöndör, K., and Thoumine, O. (2013). Biophysical mechanisms regulating AMPA receptor accumulation at synapses. *Brain Research Bulletin* 93, 57-68. doi: https://doi.org/10.1016/j.brainresbull.2012.11.001.

D. Burgoyne, R. (2004). The neuronal calcium-sensor proteins. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1742(1), 59-68. doi: https://doi.org/10.1016/j.bbamcr.2004.08.008.

d'Anglemont de Tassigny, X., Campagne, C., Dehouck, B., Leroy, D., Holstein, G.R., Beauvillain, J.C., et al. (2007). Coupling of neuronal nitric oxide synthase to NMDA receptors via postsynaptic density-95 depends on estrogen and contributes to the central control of adult female reproduction. *J Neurosci* 27(23), 6103-6114. doi: 10.1523/jneurosci.5595-06.2007.

D'Mello, R., Marchand, F., Pezet, S., McMahon, S.B., and Dickenson, A.H. (2011). Perturbing PSD-95 Interactions With NR2B-subtype Receptors Attenuates Spinal Nociceptive Plasticity and Neuropathic Pain. *Molecular Therapy* 19(10), 1780-1792. doi: 10.1038/mt.2011.42.

Dalby, N.O., and Mody, I. (2003). Activation of NMDA receptors in rat dentate gyrus granule cells by spontaneous and evoked transmitter release. *J Neurophysiol* 90(2), 786-797. doi: 10.1152/jn.00118.2003.

Dana, H., Chalbatani, G.M., Mahmoodzadeh, H., Karimloo, R., Rezaiean, O., Moradzadeh, A., et al. (2017). Molecular Mechanisms and Biological Functions of siRNA. *International journal of biomedical science : IJBS* 13(2), 48-57.

Danchenko, M., Csaderova, L., Fournier, P.E., and Sekeyova, Z. (2019). Optimized fixation of actin filaments for improved indirect immunofluorescence staining of rickettsiae. *BMC Research Notes* 12(1), 657. doi: 10.1186/s13104-019-4699-9.

Das, S., Moon, H.C., Singer, R.H., and Park, H.Y. (2018). A transgenic mouse for imaging activity-dependent dynamics of endogenous Arc mRNA in live neurons. *Science advances* 4(6), eaar3448-eaar3448. doi: 10.1126/sciadv.aar3448.

Das, U., Scott, D.A., Ganguly, A., Koo, E.H., Tang, Y., and Roy, S. (2013). Activity-induced convergence of APP and BACE-1 in acidic microdomains via an endocytosis-dependent pathway. *Neuron* 79(3), 447-460. doi: 10.1016/j.neuron.2013.05.035.

Dasgupta, A., Baby, N., Krishna, K., Hakim, M., Wong, Y.P., Behnisch, T., et al. (2017). Substance P induces plasticity and synaptic tagging/capture in rat hippocampal area CA2. *Proc Natl Acad Sci U S A* 114(41), E8741-e8749. doi: 10.1073/pnas.1711267114.

DaSilva, L.L., Wall, M.J., L, P.d.A., Wauters, S.C., Januário, Y.C., Müller, J., et al. (2016). Activity-Regulated Cytoskeleton-Associated Protein Controls AMPAR Endocytosis through a Direct Interaction with Clathrin-Adaptor Protein 2. *eNeuro* 3(3). doi: 10.1523/eneuro.0144-15.2016.

Daw, M.I., Chittajallu, R., Bortolotto, Z.A., Dev, K.K., Duprat, F., Henley, J.M., et al. (2000). PDZ Proteins Interacting with C-Terminal GluR2/3 Are Involved in a PKC-Dependent Regulation of AMPA Receptors at Hippocampal Synapses. *Neuron* 28(3), 873-886. doi: 10.1016/S0896-6273(00)00160-4.

Dawes, B.E., Gao, J., Atkins, C., Nelson, J.T., Johnson, K., Wu, P., et al. (2018). Human neural stem cell-derived neuron/astrocyte co-cultures respond to La Crosse virus infection with proinflammatory cytokines and chemokines. *Journal of Neuroinflammation* 15(1), 315. doi: 10.1186/s12974-018-1356-5.

Dawson, G.R., Seabrook, G.R., Zheng, H., Smith, D.W., Graham, S., O'Dowd, G., et al. (1999). Age-related cognitive deficits, impaired long-term potentiation and reduction in synaptic marker density in mice lacking the beta-amyloid precursor protein. *Neuroscience* 90(1), 1-13. doi: 10.1016/s0306-4522(98)00410-2.

Dayan, P., and Willshaw, D.J. (1991). Optimising synaptic learning rules in linear associative memories. *Biol Cybern* 65(4), 253-265.

de Fougerolles, A., Vornlocher, H.-P., Maraganore, J., and Lieberman, J. (2007). Interfering with disease: a progress report on siRNA-based therapeutics. *Nature Reviews Drug Discovery* 6(6), 443-453. doi: 10.1038/nrd2310.

de Oliveira Coelho, C.A., Ferreira, T.L., Soares, J.C., and Oliveira, M.G. (2013). Hippocampal NMDA receptor blockade impairs CREB phosphorylation in amygdala after contextual fear conditioning. *Hippocampus* 23(7), 545-551. doi: 10.1002/hipo.22118.

de Solis, C.A., Morales, A.A., Hosek, M.P., Partin, A.C., and Ploski, J.E. (2017). Is Arc mRNA Unique: A Search for mRNAs That Localize to the Distal Dendrites of Dentate Gyrus Granule Cells Following Neural Activity. *Frontiers in Molecular Neuroscience* 10(314). doi: 10.3389/fnmol.2017.00314.

De Strooper, B., Vassar, R., and Golde, T. (2010). The secretases: enzymes with therapeutic potential in Alzheimer disease. *Nature reviews. Neurology* 6(2), 99-107. doi: 10.1038/nrneurol.2009.218.

Debanne, D. (2009). Plasticity of neuronal excitability in vivo. *The Journal of physiology* 587(Pt 13), 3057-3058. doi: 10.1113/jphysiol.2009.175448.

Del Turco, D., Paul, M.H., Schlaudraff, J., Hick, M., Endres, K., Müller, U.C., et al. (2016). Region-Specific Differences in Amyloid Precursor Protein Expression in the Mouse Hippocampus. *Frontiers in molecular neuroscience* 9, 134-134. doi: 10.3389/fnmol.2016.00134.

Delibas, N., Doguc, D.K., Sutcu, R., Eroglu, E., and GÖKalp, O. (2005). EFFECT OF NICOTINE ON HIPPOCAMPAL NICOTINIC ACETYLCHOLINE α7 RECEPTOR AND NMDA RECEPTOR SUBUNITS 2A AND 2B EXPRESSION IN YOUNG AND OLD RATS. *International Journal of Neuroscience* 115(8), 1151-1163. doi: 10.1080/00207450590914437.

Delivopoulos, E., and Murray, A.F. (2011). Controlled adhesion and growth of long term glial and neuronal cultures on Parylene-C. *PLoS One* 6(9), e25411. doi: 10.1371/journal.pone.0025411.

Demars, M.P., Bartholomew, A., Strakova, Z., and Lazarov, O. (2011). Soluble amyloid precursor protein: a novel proliferation factor of adult progenitor cells of ectodermal and mesodermal origin. *Stem Cell Res Ther* 2(4), 36. doi: 10.1186/scrt77.

Denninger, J.W., and Marletta, M.A. (1999). Guanylate cyclase and the ·NO/cGMP signaling pathway. *Biochimica et Biophysica Acta (BBA) - Bioenergetics* 1411(2), 334-350. doi: https://doi.org/10.1016/S0005-2728(99)00024-9.

Derkach, V., Barria, A., and Soderling, T.R. (1999). Ca^{2+}/calmodulin-kinase II enhances channel conductance of alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionate type glutamate receptors. *Proc Natl Acad Sci U S A* 96(6), 3269-3274. doi: 10.1073/pnas.96.6.3269.

Di Liegro, I., Savettieri, G., Coppolino, M., Scaturro, M., Monte, M., Nastasi, T., et al. (1995). Expression of synapsin I gene in primary cultures of differentiating rat cortical neurons. *Neurochem Res* 20(2), 239-243.

Díaz-Alonso, J., Sun, Y.J., Granger, A.J., Levy, J.M., Blankenship, S.M., and Nicoll, R.A. (2017). Subunit-specific role for the amino-terminal domain of AMPA receptors in synaptic targeting.

Proceedings of the National Academy of Sciences 114(27), 7136-7141. doi: 10.1073/pnas.1707472114.

Dichter, M.A., Lisak, J., and Biales, B. (1983). Action potential mechanism of mammalian cortical neurons in cell culture. *Brain Res* 289(1-2), 99-107. doi: 10.1016/0006-8993(83)90010-0.

Didier, S., Sauvé, F., Domise, M., Buée, L., Marinangeli, C., and Vingtdeux, V. (2018). AMP-activated Protein Kinase Controls Immediate Early Genes Expression Following Synaptic Activation Through the PKA/CREB Pathway. *International journal of molecular sciences* 19(12), 3716. doi: 10.3390/ijms19123716.

Diederich, K., Sevimli, S., Dörr, H., Kösters, E., Hoppen, M., Lewejohann, L., et al. (2009). The role of granulocyte-colony stimulating factor (G-CSF) in the healthy brain: a characterization of G-CSF-deficient mice. *J Neurosci* 29(37), 11572-11581. doi: 10.1523/jneurosci.0453-09.2009.

Diering, G.H., and Huganir, R.L. (2018). The AMPA Receptor Code of Synaptic Plasticity. *Neuron* 100(2), 314-329. doi: 10.1016/j.neuron.2018.10.018.

Diering, G.H., Gustina, A.S., and Huganir, R.L. (2014). PKA-GluA1 coupling via AKAP5 controls AMPA receptor phosphorylation and cell-surface targeting during bidirectional homeostatic plasticity. *Neuron* 84(4), 790-805. doi: 10.1016/j.neuron.2014.09.024.

Diering, G.H., Heo, S., Hussain, N.K., Liu, B., and Huganir, R.L. (2016). Extensive phosphorylation of AMPA receptors in neurons. *Proceedings of the National Academy of Sciences* 113(33), E4920-E4927. doi: 10.1073/pnas.1610631113.

Diering, G.H., Nirujogi, R.S., Roth, R.H., Worley, P.F., Pandey, A., and Huganir, R.L. (2017). Homer1a drives homeostatic scaling-down of excitatory synapses during sleep. *Science (New York, N.Y.)* 355(6324), 511-515. doi: 10.1126/science.aai8355.

Dieterich, D.C., Hodas, J.J.L., Gouzer, G., Shadrin, I.Y., Ngo, J.T., Triller, A., et al. (2010). In situ visualization and dynamics of newly synthesized proteins in rat hippocampal neurons. *Nat Neurosci* 13(7), 897-905. doi: 10.1038/nn.2580.

Digilio, L., Yap, C.C., and Winckler, B. (2015). Ctip2-, Satb2-, Prox1-, and GAD65-Expressing Neurons in Rat Cultures: Preponderance of Single- and Double-Positive Cells, and Cell Type-Specific Expression of Neuron-Specific Gene Family Members, Nsg-1 (NEEP21) and Nsg-2 (P19). *PLOS ONE* 10(10), e0140010. doi: 10.1371/journal.pone.0140010.

Dineley, K.T., Bell, K.A., Bui, D., and Sweatt, J.D. (2002). beta -Amyloid peptide activates alpha 7 nicotinic acetylcholine receptors expressed in Xenopus oocytes. *J Biol Chem* 277(28), 25056-25061. doi: 10.1074/jbc.M200066200.

Ding, Q., Markesbery, W.R., Chen, Q., Li, F., and Keller, J.N. (2005). Ribosome dysfunction is an early event in Alzheimer's disease. *J Neurosci* 25(40), 9171-9175. doi: 10.1523/jneurosci.3040-05.2005.

Dini, L., Coppola, S., Ruzittu, M.T., and Ghibelli, L. (1996). Multiple pathways for apoptotic nuclear fragmentation. *Exp Cell Res* 223(2), 340-347. doi: 10.1006/excr.1996.0089.

Dodonova, S.O., Prinz, S., Bilanchone, V., Sandmeyer, S., and Briggs, J.A.G. (2019). Structure of the Ty3/Gypsy retrotransposon capsid and the evolution of retroviruses. *Proceedings of the National Academy of Sciences* 116(20), 10048-10057. doi: 10.1073/pnas.1900931116.

Dodt, H.-U., Frick, A., Kampe, K., and Zieglgänsberger, W. (1998). NMDA and AMPA receptors on neocortical neurons are differentially distributed. *European Journal of Neuroscience* 10(11), 3351-3357. doi: 10.1046/j.1460-9568.1998.00338.x.

Dominguez del Toro, E., Juiz, J.M., Peng, X., Lindstrom, J., and Criado, M. (1994). Immunocytochemical localization of the alpha 7 subunit of the nicotinic acetylcholine receptor in the rat central nervous system. *J Comp Neurol* 349(3), 325-342. doi: 10.1002/cne.903490302.

Donai, H., Sugiura, H., Ara, D., Yoshimura, Y., Yamagata, K., and Yamauchi, T. (2003). Interaction of Arc with CaM kinase II and stimulation of neurite extension by Arc in neuroblastoma cells expressing CaM kinase II. *Neurosci Res* 47(4), 399-408. doi: 10.1016/j.neures.2003.08.004.

Dong, H., O'Brien, R.J., Fung, E.T., Lanahan, A.A., Worley, P.F., and Huganir, R.L. (1997). GRIP: a synaptic PDZ domain-containing protein that interacts with AMPA receptors. *Nature* 386(6622), 279-284. doi: 10.1038/386279a0.

Dong, L.Q., Landa, L.R., Wick, M.J., Zhu, L., Mukai, H., Ono, Y., et al. (2000). Phosphorylation of protein kinase N by phosphoinositide-dependent protein kinase-1 mediates insulin signals to the actin cytoskeleton. *Proc Natl Acad Sci U S A* 97(10), 5089-5094. doi: 10.1073/pnas.090491897.

Dong, Y. (2016). Silent Synapse-Based Circuitry Remodeling in Drug Addiction. *The international journal of neuropsychopharmacology* 19(5), pyv136. doi: 10.1093/ijnp/pyv136.

Doody, R.S., Thomas, R.G., Farlow, M., Iwatsubo, T., Vellas, B., Joffe, S., et al. (2014). Phase 3 trials of solanezumab for mild-to-moderate Alzheimer's disease. *N Engl J Med* 370(4), 311-321. doi: 10.1056/NEJMoa1312889.

Dorard, E., Chasseigneaux, S., Gorisse-Hussonnois, L., Broussard, C., Pillot, T., and Allinquant, B. (2018). Soluble Amyloid Precursor Protein Alpha Interacts with alpha3-Na, K-ATPAse to Induce Axonal Outgrowth but Not Neuroprotection: Evidence for Distinct Mechanisms Underlying these Properties. *Mol Neurobiol* 55(7), 5594-5610. doi: 10.1007/s12035-017-0783-0.

Dotti, C.G., Sullivan, C.A., and Banker, G.A. (1988). The establishment of polarity by hippocampal neurons in culture. *J Neurosci* 8(4), 1454-1468. doi: 10.1523/jneurosci.08-04-01454.1988.

Dougherty, J.J., Wu, J., and Nichols, R.A. (2003). Beta-amyloid regulation of presynaptic nicotinic receptors in rat hippocampus and neocortex. *J Neurosci* 23(17), 6740-6747. doi: 10.1523/jneurosci.23-17-06740.2003.

Doyle, E., Bruce, M.T., Breen, K.C., Smith, D.C., Anderton, B., and Regan, C.M. (1990). Intraventricular infusions of antibodies to amyloid-beta-protein precursor impair the acquisition of a passive avoidance response in the rat. *Neurosci Lett* 115(1), 97-102. doi: 10.1016/0304-3940(90)90524-d.

Dreyer, J.-L. (2010). "Lentiviral Vector-Mediated Gene Transfer and RNA Silencing Technology in Neuronal Dysfunctions," in *Lentivirus Gene Engineering Protocols: Second Edition,* ed. M. Federico. (Totowa, NJ: Humana Press), 3-35.

Duce, J.A., Tsatsanis, A., Cater, M.A., James, S.A., Robb, E., Wikhe, K., et al. (2010). Iron-export ferroxidase activity of β-amyloid precursor protein is inhibited by zinc in Alzheimer's disease. *Cell* 142(6), 857-867. doi: 10.1016/j.cell.2010.08.014.

Dudek, S.M., and Bear, M.F. (1992). Homosynaptic long-term depression in area CA1 of hippocampus and effects of N-methyl-D-aspartate receptor blockade. *Proceedings of the National Academy of Sciences of the United States of America* 89(10), 4363-4367. doi: 10.1073/pnas.89.10.4363.

Dumas, T.C., Uttaro, M.R., Barriga, C., Brinkley, T., Halavi, M., Wright, S.N., et al. (2018). Removal of area CA3 from hippocampal slices induces postsynaptic plasticity at Schaffer collateral synapses that normalizes CA1 pyramidal cell discharge. *Neuroscience letters* 678, 55-61. doi: 10.1016/j.neulet.2018.05.011.

Dunwiddie, T.V., and Lynch, G. (1979). The relationship between extracellular calcium concentrations and the induction of hippocampal long-term potentiation. *Brain Res* 169(1), 103-110.

Durand, G.M., Kovalchuk, Y., and Konnerth, A. (1996). Long-term potentiation and functional synapse induction in developing hippocampus. *Nature* 381(6577), 71-75. doi: 10.1038/381071a0.

Dynes, J.L., and Steward, O. (2007). Dynamics of bidirectional transport of Arc mRNA in neuronal dendrites. *The Journal of Comparative Neurology* 500(3), 433-447. doi: 10.1002/cne.21189.

Dynes, J.L., and Steward, O. (2012). Arc mRNA docks precisely at the base of individual dendritic spines indicating the existence of a specialized microdomain for synapse-specific mRNA translation. *J Comp Neurol* 520(14), 3105-3119. doi: 10.1002/cne.23073.

Dzyubenko, E., Rozenberg, A., Hermann, D.M., and Faissner, A. (2016). Colocalization of synapse marker proteins evaluated by STED-microscopy reveals patterns of neuronal synapse distribution in vitro. *J Neurosci Methods* 273, 149-159. doi: 10.1016/j.jneumeth.2016.09.001.

Earnshaw, B.A., and Bressloff, P.C. (2008). Modeling the role of lateral membrane diffusion in AMPA receptor trafficking along a spiny dendrite. *J Comput Neurosci* 25(2), 366-389. doi: 10.1007/s10827-008-0084-8.

Ebbinghaus, H. (1885). *Über das gedächtnis: untersuchungen zur experimentellen psychologie.* Duncker & Humblot.

Eccles, J.C. (1983). Calcium in long-term potentiation as a model for memory. *Neuroscience* 10(4), 1071-1081. doi: https://doi.org/10.1016/0306-4522(83)90100-8.

Edelmann, E., Cepeda-Prado, E., and Leßmann, V. (2017). Coexistence of Multiple Types of Synaptic Plasticity in Individual Hippocampal CA1 Pyramidal Neurons. *Frontiers in Synaptic Neuroscience* 9(7). doi: 10.3389/fnsyn.2017.00007.

Edwards, F.A., and Konnerth, A. (1992). Patch-clamping cells in sliced tissue preparations. *Methods Enzymol* 207, 208-222. doi: 10.1016/0076-6879(92)07015-g.

Egan, M.F., Kost, J., Tariot, P.N., Aisen, P.S., Cummings, J.L., Vellas, B., et al. (2018). Randomized Trial of Verubecestat for Mild-to-Moderate Alzheimer's Disease. *N Engl J Med* 378(18), 1691-1703. doi: 10.1056/NEJMoa1706441.

Eggert, S., Paliga, K., Soba, P., Evin, G., Masters, C.L., Weidemann, A., et al. (2004). The Proteolytic Processing of the Amyloid Precursor Protein Gene Family Members APLP-1 and APLP-2 Involves α-, β-, γ-, and ϵ-Like Cleavages: MODULATION OF APLP-1 PROCESSING BY N-GLYCOSYLATION. *Journal of Biological Chemistry* 279(18), 18146-18156. doi: 10.1074/jbc.M311601200.

Ehlers, M.D. (2000). Reinsertion or Degradation of AMPA Receptors Determined by Activity-Dependent Endocytic Sorting. *Neuron* 28(2), 511-525. doi: https://doi.org/10.1016/S0896-6273(00)00129-X.

Ehlers, M.D., Heine, M., Groc, L., Lee, M.-C., and Choquet, D. (2007). Diffusional trapping of GluR1 AMPA receptors by input-specific synaptic activity. *Neuron* 54(3), 447-460. doi: 10.1016/j.neuron.2007.04.010.

Ehrlich, I., and Malinow, R. (2004). Postsynaptic density 95 controls AMPA receptor incorporation during long-term potentiation and experience-driven synaptic plasticity. *J Neurosci* 24(4), 916-927. doi: 10.1523/jneurosci.4733-03.2004.

Ehrlich, I., Klein, M., Rumpel, S., and Malinow, R. (2007). PSD-95 is required for activity-driven synapse stabilization. *Proceedings of the National Academy of Sciences* 104(10), 4176-4181. doi: 10.1073/pnas.0609307104.

Eide, L., and McMurray, C.T. (2005). Culture of adult mouse neurons. *BioTechniques* 38(1), 99-104. doi: 10.2144/05381rr02.

Eketjäll, S., Janson, J., Kaspersson, K., Bogstedt, A., Jeppsson, F., Fälting, J., et al. (2016). AZD3293: A Novel, Orally Active BACE1 Inhibitor with High Potency and Permeability and Markedly Slow Off-Rate Kinetics. *Journal of Alzheimer's disease : JAD* 50(4), 1109-1123. doi: 10.3233/JAD-150834.

El-Husseini, A.E.-D., Schnell, E., Chetkovich, D.M., Nicoll, R.A., and Bredt, D.S. (2000). PSD-95 Involvement in Maturation of Excitatory Synapses. *Science* 290(5495), 1364-1368. doi: 10.1126/science.290.5495.1364.

El-Sayed, M., Hofman-Bang, J., and Mikkelsen, J.D. (2011). Effect of brain-derived neurotrophic factor on activity-regulated cytoskeleton-associated protein gene expression in primary frontal cortical neurons. Comparison with NMDA and AMPA. *Eur J Pharmacol* 660(2-3), 351-357. doi: 10.1016/j.ejphar.2011.03.055.

Elamri, I., Heumüller, M., Herzig, L.-M., Stirnal, E., Wachtveitl, J., Schuman, E.M., et al. (2018). A New Photocaged Puromycin for an Efficient Labeling of Newly Translated Proteins in Living Neurons. *ChemBioChem* 19(23), 2458-2464. doi: 10.1002/cbic.201800408.

Elder, M.K. (2017) Secreted amyloid precursor protein alpha regulates AMPA receptor synthesis and expression. *PhD Thesis*, Dunedin: University of Otago. Retrieved from http://hdl.handle.net/10523/7692.

Elias, G.M., and Nicoll, R.A. (2007). Synaptic trafficking of glutamate receptors by MAGUK scaffolding proteins. *Trends in Cell Biology* 17(7), 343-352. doi: https://doi.org/10.1016/j.tcb.2007.07.005.

Ellenbroek, B., and Youn, J. (2016). Rodent models in neuroscience research: is it a rat race? *Disease models & mechanisms* 9(10), 1079-1087. doi: 10.1242/dmm.026120.

Endo, S., and Launey, T. (2003). Nitric oxide activates extracellular signal-regulated kinase 1/2 and enhances declustering of ionotropic glutamate receptor subunit 2/3 in rat cerebellar Purkinje cells. *Neuroscience Letters* 350(2), 122-126. doi: https://doi.org/10.1016/S0304-3940(03)00856-5.

Eng, L.F., Ghirnikar, R.S., and Lee, Y.L. (2000). Glial fibrillary acidic protein: GFAP-thirty-one years (1969-2000). *Neurochem Res* 25(9-10), 1439-1451.

English, J.A., Fan, Y., Föcking, M., Lopez, L.M., Hryniewiecka, M., Wynne, K., et al. (2015). Reduced protein synthesis in schizophrenia patient-derived olfactory cells. *Translational psychiatry* 5(10), e663-e663. doi: 10.1038/tp.2015.119.

Enkvist, M.O., Holopainen, I., and Akerman, K.E. (1989). Glutamate receptor-linked changes in membrane potential and intracellular Ca^{2+} in primary rat astrocytes. *Glia* 2(6), 397-402. doi: 10.1002/glia.440020602.

Enriquez-Barreto, L., Cuesto, G., Dominguez-Iturza, N., Gavilán, E., Ruano, D., Sandi, C., et al. (2014). Learning improvement after PI3K activation correlates with de novo formation of functional small spines. Frontiers in molecular neuroscience 6, 54-54. doi: 10.3389/fnmol.2013.00054.

Ertekin-Taner, N. (2010). Genetics of Alzheimer disease in the pre- and post-GWAS era. *Alzheimer's Research & Therapy* 2(1), 3. doi: 10.1186/alzrt26.

Eskiw, C.H., and Bazett-Jones, D.P. (2002). The promyelocytic leukemia nuclear body: sites of activity? *Biochem Cell Biol* 80(3), 301-310. doi: 10.1139/o02-079.

Esteban, J.A., Shi, S.-H., Wilson, C., Nuriya, M., Huganir, R.L., and Malinow, R. (2003). PKA phosphorylation of AMPA receptor subunits controls synaptic trafficking underlying plasticity. *Nature Neuroscience* 6(2), 136-143. doi: 10.1038/nn997.

Evans, H.T., Benetatos, J., van Roijen, M., Bodea, L.-G., and Götz, J. (2019). Decreased synthesis of ribosomal proteins in tauopathy revealed by non-canonical amino acid labelling. The EMBO journal 38(13), e101174-e101174. doi: 10.15252/embj.2018101174.

Evonuk, K.S., Doyle, R.E., Moseley, C.E., Thornell, I.M., Adler, K., Bingaman, A.M., et al. (2020). Reduction of AMPA receptor activity on mature oligodendrocytes attenuates loss of myelinated axons in autoimmune neuroinflammation. *Science Advances* 6(2), eaax5936. doi: 10.1126/sciadv.aax5936.

Eyjolfsdottir, H., Eriksdotter, M., Linderoth, B., Lind, G., Juliusson, B., Kusk, P., et al. (2016). Targeted delivery of nerve growth factor to the cholinergic basal forebrain of Alzheimer's disease patients: application of a second-generation encapsulated cell biodelivery device. *Alzheimer's Research & Therapy* 8(1), 30. doi: 10.1186/s13195-016-0195-9.

Fachim, H.A., Pereira, A.C., Iyomasa-Pilon, M.M., and Rosa, M.L.N.M. (2016). Differential Expression of AMPA Subunits Induced by NMDA Intrahippocampal Injection in Rats. *Frontiers in Neuroscience* 10(32). doi: 10.3389/fnins.2016.00032.

Farlow, M., Arnold, S.E., van Dyck, C.H., Aisen, P.S., Snider, B.J., Porsteinsson, A.P., et al. (2012). Safety and biomarker effects of solanezumab in patients with Alzheimer's disease. *Alzheimers Dement* 8(4), 261-271. doi: 10.1016/j.jalz.2011.09.224.

Farris, S., Lewandowski, G., Cox, C.D., and Steward, O. (2014). Selective Localization of Arc mRNA in Dendrites Involves Activity- and Translation-Dependent mRNA Degradation. *J Neurosci* 34(13), 4481-4493. doi: 10.1523/jneurosci.4944-13.2014.

Fasano, C., Rocchetti, J., Pietrajtis, K., Zander, J.-F., Manseau, F., Sakae, D.Y., et al. (2017). Regulation of the Hippocampal Network by VGLUT3-Positive CCK- GABAergic Basket Cells. *Frontiers in Cellular Neuroscience* 11(140). doi: 10.3389/fncel.2017.00140.

Faucher, P., Mons, N., Micheau, J., Louis, C., and Beracochea, D.J. (2016). Hippocampal Injections of Oligomeric Amyloid β-peptide (1–42) Induce Selective Working Memory Deficits and Long-

lasting Alterations of ERK Signaling Pathway. *Frontiers in Aging Neuroscience* 7(245). doi: 10.3389/fnagi.2015.00245.

Fayuk, D., and Yakel, J.L. (2007). Dendritic Ca^{2+} signalling due to activation of alpha 7-containing nicotinic acetylcholine receptors in rat hippocampal neurons. *The Journal of physiology* 582(Pt 2), 597-611. doi: 10.1113/jphysiol.2007.135319.

Fazeli, M.S., Corbet, J., Dunn, M.J., Dolphin, A.C., and Bliss, T.V. (1993). Changes in protein synthesis accompanying long-term potentiation in the dentate gyrus in vivo. *J Neurosci* 13(4), 1346-1353.

Feil, R., Hartmann, J., Luo, C., Wolfsgruber, W., Schilling, K., Feil, S., et al. (2003). Impairment of LTD and cerebellar learning by Purkinje cell–specific ablation of cGMP-dependent protein kinase I. *The Journal of Cell Biology* 163(2), 295-302. doi: 10.1083/jcb.200306148.

Fioriti, L., Myers, C., Huang, Y.-Y., Li, X., Stephan, Joseph S., Trifilieff, P., et al. (2015). The Persistence of Hippocampal-Based Memory Requires Protein Synthesis Mediated by the Prion-like Protein CPEB3. *Neuron* 86(6), 1433-1448. doi: https://doi.org/10.1016/j.neuron.2015.05.021.

Fisher, A. (2012). Cholinergic modulation of amyloid precursor protein processing with emphasis on M1 muscarinic receptor: perspectives and challenges in treatment of Alzheimer's disease. *J Neurochem* 120 Suppl 1, 22-33. doi: 10.1111/j.1471-4159.2011.07507.x.

Fletcher, T.L., Cameron, P., De Camilli, P., and Banker, G. (1991). The distribution of synapsin I and synaptophysin in hippocampal neurons developing in culture. *J Neurosci* 11(6), 1617-1626. doi: 10.1523/jneurosci.11-06-01617.1991.

Fletcher, T.L., De Camilli, P., and Banker, G. (1994). Synaptogenesis in hippocampal cultures: evidence indicating that axons and dendrites become competent to form synapses at different stages of neuronal development. *J Neurosci* 14(11 Pt 1), 6695-6706.

Flexner, J.B., Flexner, L.B., and Stellar, E. (1963). Memory in mice as affected by intracerebral puromycin. *Science* 141(3575), 57-59.

Flexner, J.B., Flexner, L.B., Stellar, E., De La Haba, G., and Roberts, R.B. (1962). Inhibition of protein synthesis in brain and learning and memory following puromycin. *J Neurochem* 9, 595-605.

Flexner, L.B., Flexner, J.B., and Roberts, R.B. (1966). Stages of memory in mice treated with acetoxycycloheximide before or immediately after learning. *Proc Natl Acad Sci U S A* 56(2), 730-735.

Flexner, L.B., Flexner, J.B., and Stellar, E. (1965). Memory and cerebral protein synthesis in mice as affected by graded amounts of puromycin. *Exp Neurol* 13(3), 264-272.

Fol, R., Braudeau, J., Ludewig, S., Abel, T., Weyer, S.W., Roederer, J.P., et al. (2016). Viral gene transfer of APPsalpha rescues synaptic failure in an Alzheimer's disease mouse model. *Acta Neuropathol* 131(2), 247-266. doi: 10.1007/s00401-015-1498-9.

Forest, K.H., Alfulaij, N., Arora, K., Taketa, R., Sherrin, T., Todorovic, C., et al. (2018). Protection against β-amyloid neurotoxicity by a non-toxic endogenous N-terminal β-amyloid fragment and its active hexapeptide core sequence. *Journal of Neurochemistry* 144(2), 201-217. doi: doi:10.1111/jnc.14257.

Forman, M., Kleijn, H.-J., Dockendorf, M., Palcza, J., Tseng, J., Canales, C., et al. (2013). The novel BACE inhibitor MK-8931 dramatically lowers CSF beta-amyloid in patients with mild-to-moderate Alzheimer's disease. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association* 9(4), P139. doi: 10.1016/j.jalz.2013.04.083.

Forsythe, I.D., Tsujimoto, T., Barnes-Davies, M., Cuttle, M.F., and Takahashi, T. (1998). Inactivation of presynaptic calcium current contributes to synaptic depression at a fast central synapse. *Neuron* 20(4), 797-807.

Fortin, D.A., Davare, M.A., Srivastava, T., Brady, J.D., Nygaard, S., Derkach, V.A., et al. (2010). Long-term potentiation-dependent spine enlargement requires synaptic Ca^{2+}-permeable AMPA receptors recruited by CaM-kinase I. *J Neurosci* 30(35), 11565-11575. doi: 10.1523/jneurosci.1746-10.2010.

Fortin, D.A., Srivastava, T., Dwarakanath, D., Pierre, P., Nygaard, S., Derkach, V.A., et al. (2012). Brain-Derived Neurotrophic Factor Activation of CaM-Kinase Kinase via Transient Receptor Potential

Canonical Channels Induces the Translation and Synaptic Incorporation of GluA1-Containing Calcium-Permeable AMPA Receptors. *The Journal of Neuroscience* 32(24), 8127-8137. doi: 10.1523/jneurosci.6034-11.2012.

Fosnaugh, J.S., Bhat, R.V., Yamagata, K., Worley, P.F., and Baraban, J.M. (1995). Activation of arc, a putative "effector" immediate early gene, by cocaine in rat brain. *J Neurochem* 64(5), 2377-2380. doi: 10.1046/j.1471-4159.1995.64052377.x.

Frazier, C.J., Strowbridge, B.W., and Papke, R.L. (2003). Nicotinic Receptors on Local Circuit Neurons in Dentate Gyrus: A Potential Role in Regulation of Granule Cell Excitability. *Journal of Neurophysiology* 89(6), 3018-3028. doi: 10.1152/jn.01036.2002.

Freir, D.B., Holscher, C., and Herron, C.E. (2001). Blockade of long-term potentiation by beta-amyloid peptides in the CA1 region of the rat hippocampus in vivo. *J Neurophysiol* 85(2), 708-713. doi: 10.1152/jn.2001.85.2.708.

Freud, S., and Breuer, J. (1895). Studien über Hysterie (Studies on Hysteria). *Leipzig: Franz Deuticke*.

Freude, K.K., Penjwini, M., Davis, J.L., LaFerla, F.M., and Blurton-Jones, M. (2011). Soluble amyloid precursor protein induces rapid neural differentiation of human embryonic stem cells. *J Biol Chem* 286(27), 24264-24274. doi: 10.1074/jbc.M111.227421.

Freund TF, Buzsáki G (1996) Interneurons of the hippocampus. Hippocampus 6:347-470.

Frey, S., Bergado-Rosado, J., Seidenbecher, T., Pape, H.-C., and Frey, J.U. (2001). Reinforcement of Early Long-Term Potentiation (Early-LTP) in Dentate Gyrus by Stimulation of the Basolateral Amygdala: Heterosynaptic Induction Mechanisms of Late-LTP. *The Journal of Neuroscience* 21(10), 3697-3703. doi: 10.1523/jneurosci.21-10-03697.2001.

Frey, S., Schwiegert, C., Krug, M., and Lössner, B. (1991a). Long-term potentiation induced changes in protein synthesis of hippocampal subfields of freely moving rats: time-course. *Biomedica biochimica acta* 50(12), 1231-1240.

Frey, U., Frey, S., Schollmeier, F., and Krug, M. (1996). Influence of actinomycin D, a RNA synthesis inhibitor, on long-term potentiation in rat hippocampal neurons in vivo and in vitro. *J Physiol* 490 (Pt 3), 703-711.

Frey, U., Huang, Y., and Kandel, E. (1993). Effects of cAMP simulate a late stage of LTP in hippocampal CA1 neurons. *Science* 260(5114), 1661-1664. doi: 10.1126/science.8389057.

Frey, U., Krug, M., Brödemann, R., Reymann, K., and Matthies, H. (1989). Long-term potentiation induced in dendrites separated from rat's CA1 pyramidal somata does not establish a late phase. *Neuroscience Letters* 97(1), 135-139. doi: https://doi.org/10.1016/0304-3940(89)90152-3.

Frey, U., Krug, M., Reymann, K.G., and Matthies, H. (1988). Anisomycin, an inhibitor of protein synthesis, blocks late phases of LTP phenomena in the hippocampal CA1 region in vitro. *Brain Research* 452(1), 57-65. doi: https://doi.org/10.1016/0006-8993(88)90008-X.

Frey, U., Matthies, H., Reymann, K.G., and Matthies, H. (1991b). The effect of dopaminergic D1 receptor blockade during tetanization on the expression of long-term potentiation in the rat CA1 region in vitro. *Neuroscience letters* 129(1), 111-114.

Frotscher, M., and Deller, T. (2005). "Role of the Spine Apparatus in Synaptic Plasticity," in *Synaptic Plasticity and Transsynaptic Signaling,* eds. P.K. Stanton, C. Bramham & H.E. Scharfman. (Boston, MA: Springer US), 519-528.

Frykman, S., Hur, J.-Y., Frånberg, J., Aoki, M., Winblad, B., Nahalkova, J., et al. (2010). Synaptic and Endosomal Localization of Active γ-Secretase in Rat Brain. *PLOS ONE* 5(1), e8948. doi: 10.1371/journal.pone.0008948.

Fujimoto, T., Tanaka, H., Kumamaru, E., Okamura, K., and Miki, N. (2004). Arc interacts with microtubules/microtubule-associated protein 2 and attenuates microtubule-associated protein 2 immunoreactivity in the dendrites. *Journal of Neuroscience Research* 76(1), 51-63. doi: 10.1002/jnr.20056.

Fukazawa, Y., Saitoh, Y., Ozawa, F., Ohta, Y., Mizuno, K., and Inokuchi, K. (2003). Hippocampal LTP is accompanied by enhanced F-actin content within the dendritic spine that is essential for late LTP maintenance in vivo. *Neuron* 38(3), 447-460.

Fukumoto, H., Cheung, B.S., Hyman, B.T., and Irizarry, M.C. (2002). Beta-secretase protein and activity are increased in the neocortex in Alzheimer disease. *Arch Neurol* 59(9), 1381-1389. doi: 10.1001/archneur.59.9.1381.

Fukumoto, H., Rosene, D.L., Moss, M.B., Raju, S., Hyman, B.T., and Irizarry, M.C. (2004). Beta-secretase activity increases with aging in human, monkey, and mouse brain. *Am J Pathol* 164(2), 719-725. doi: 10.1016/s0002-9440(10)63159-8.

Furini, C.R., Rossato, J.I., Bitencourt, L.L., Medina, J.H., Izquierdo, I., and Cammarota, M. (2010). Beta-adrenergic receptors link NO/sGC/PKG signaling to BDNF expression during the consolidation of object recognition long-term memory. *Hippocampus* 20(5), 672-683. doi: 10.1002/hipo.20656.

Furukawa, K., and Mattson, M.P. (1998b). Secreted amyloid precursor protein α selectively suppresses N-methyl-d-aspartate currents in hippocampal neurons: involvement of cyclic GMP. *Neuroscience* 83(2), 429-438. doi: https://doi.org/10.1016/S0306-4522(97)00398-9.

Furukawa, K., Barger, S.W., Blalock, E.M., and Mattson, M.P. (1996a). Activation of K+ channels and suppression of neuronal activity by secreted β-amyloid-precursor protein. *Nature* 379(6560), 74-78. doi: 10.1038/379074a0.

Furukawa, K., Sopher, B.L., Rydel, R.E., Begley, J.G., Pham, D.G., Martin, G.M., et al. (1996b). Increased activity-regulating and neuroprotective efficacy of alpha-secretase-derived secreted amyloid precursor protein conferred by a C-terminal heparin-binding domain. *J Neurochem* 67(5), 1882-1896.

Gainey, M.A., Hurvitz-Wolff, J.R., Lambo, M.E., and Turrigiano, G.G. (2009). Synaptic Scaling Requires the GluR2 Subunit of the AMPA Receptor. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 29(20), 6479-6489. doi: 10.1523/JNEUROSCI.3753-08.2009.

Gakhar-Koppole, N., Hundeshagen, P., Mandl, C., Weyer, S.W., Allinquant, B., Muller, U., et al. (2008). Activity requires soluble amyloid precursor protein alpha to promote neurite outgrowth in neural stem cell-derived neurons via activation of the MAPK pathway. *Eur J Neurosci* 28(5), 871-882. doi: 10.1111/j.1460-9568.2008.06398.x.

Gallo, E.F., and Iadecola, C. (2011). Neuronal Nitric Oxide Contributes to Neuroplasticity-Associated Protein Expression through cGMP, Protein Kinase G, and Extracellular Signal-Regulated Kinase. *The Journal of Neuroscience* 31(19), 6947-6955. doi: 10.1523/jneurosci.0374-11.2011.

Gamper, E. (1928). Zur Frage der Polioencephalitis haemorrhagica der chronischen Alkoholiker. Anatomische Befunde beim alkoholischen Korsakow und ihre Beziehungen zum klinischen Bild. *Deutsche Zeitschrift für Nervenheilkunde* 102(1-4), 122-129.

Ganeshina, O., Berry, R.W., Petralia, R.S., Nicholson, D.A., and Geinisman, Y. (2004). Synapses with a segmented, completely partitioned postsynaptic density express more AMPA receptors than other axospinous synaptic junctions. *Neuroscience* 125(3), 615-623. doi: 10.1016/j.neuroscience.2004.02.025.

Gao, C., Sun, X., and Wolf, M.E. (2006). Activation of D1 dopamine receptors increases surface expression of AMPA receptors and facilitates their synaptic incorporation in cultured hippocampal neurons. *Journal of Neurochemistry* 98(5), 1664-1677. doi: 10.1111/j.1471-4159.2006.03999.x.

Gao, M., Sossa, K., Song, L., Errington, L., Cummings, L., Hwang, H., et al. (2010). A specific requirement of Arc/Arg3.1 for visual experience-induced homeostatic synaptic plasticity in mouse primary visual cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 30(21), 7168-7178. doi: 10.1523/JNEUROSCI.1067-10.2010.

Gao, X., Castro-Gomez, S., Grendel, J., Graf, S., Süsens, U., Binkle, L., et al. (2018). Arc/Arg3.1 mediates a critical period for spatial learning and hippocampal networks. *Proceedings of the National Academy of Sciences* 115(49), 12531-12536. doi: 10.1073/pnas.1810125115.

Garafalo, S.D., Luth, E.S., Moss, B.J., Monteiro, M.I., Malkin, E., and Juo, P. (2015). The AP2 clathrin adaptor protein complex regulates the abundance of GLR-1 glutamate receptors in the ventral nerve cord of Caenorhabditis elegans. *Mol Biol Cell* 26(10), 1887-1900. doi: 10.1091/mbc.E14-06-1048.

Garaschuk, O., Schneggenburger, R., Schirra, C., Tempia, F., and Konnerth, A. (1996). Fractional Ca^{2+} currents through somatic and dendritic glutamate receptor channels of rat hippocampal CA1 pyramidal neurones. *J Physiol* 491(Pt 3), 757-772.

Garcia-Esparcia, P., Sideris-Lampretsas, G., Hernandez-Ortega, K., Grau-Rivera, O., Sklaviadis, T., Gelpi, E., et al. (2017). Altered mechanisms of protein synthesis in frontal cortex in Alzheimer disease and a mouse model. *American journal of neurodegenerative disease* 6(2), 15-25.

Garcia-Osta, A., and Alberini, C.M. (2009). Amyloid beta mediates memory formation. *Learn Mem* 16(4), 267-272. doi: 10.1101/lm.1310209.

Gardner, S.M., Takamiya, K., Xia, J., Suh, J.-G., Johnson, R., Yu, S., et al. (2005). Calcium-Permeable AMPA Receptor Plasticity Is Mediated by Subunit-Specific Interactions with PICK1 and NSF. *Neuron* 45(6), 903-915. doi: https://doi.org/10.1016/j.neuron.2005.02.026.

Gardner, S.M., Trussell, L.O., and Oertel, D. (1999). Time course and permeation of synaptic AMPA receptors in cochlear nuclear neurons correlate with input. *Journal of Neuroscience* 19(20), 8721-8729.

Gardner, S.M., Trussell, L.O., and Oertel, D. (2001). Correlation of AMPA receptor subunit composition with synaptic input in the mammalian cochlear nuclei. *Journal of Neuroscience* 21(18), 7428-7437.

Gardoni, F., Caputi, A., Cimino, M., Pastorino, L., Cattabeni, F., and Di Luca, M. (1998). Calcium/Calmodulin-Dependent Protein Kinase II Is Associated with NR2A/B Subunits of NMDA Receptor in Postsynaptic Densities. *Journal of Neurochemistry* 71(4), 1733-1741. doi: 10.1046/j.1471-4159.1998.71041733.x.

Gardoni, F., Polli, F., Cattabeni, F., and Di Luca, M. (2006). Calcium-calmodulin-dependent protein kinase II phosphorylation modulates PSD-95 binding to NMDA receptors. *Eur J Neurosci* 24(10), 2694-2704. doi: 10.1111/j.1460-9568.2006.05140.x.

Garthwaite, J. (1985). Cellular uptake disguises action of L-glutamate on N-methyl-D-aspartate receptors. With an appendix: diffusion of transported amino acids into brain slices. *Br J Pharmacol* 85(1), 297-307. doi: 10.1111/j.1476-5381.1985.tb08860.x.

Gasparini, S., Saviane, C., Voronin, L.L., and Cherubini, E. (2000). Silent synapses in the developing hippocampus: Lack of functional AMPA receptors or low probability of glutamate release? *Proceedings of the National Academy of Sciences* 97(17), 9741-9746. doi: 10.1073/pnas.170032297.

Ge, W.P., Yang, X.J., Zhang, Z., Wang, H.K., Shen, W., Deng, Q.D., et al. (2006). Long-term potentiation of neuron-glia synapses mediated by Ca^{2+}-permeable AMPA receptors. *Science* 312(5779), 1533-1537. doi: 10.1126/science.1124669.

Geiger, J.R.P., Melcher, T., Koh, D.S., Sakmann, B., Seeburg, P.H., Jonas, P., et al. (1995). Relative abundance of subunit mRNAs determines gating and Ca^{2+} permeability of AMPA receptors in principal neurons and interneurons in rat CNS. *Neuron* 15(1), 193-204. doi: https://doi.org/10.1016/0896-6273(95)90076-4.

Geisert, E.E., Jr., Johnson, H.G., and Binder, L.I. (1990). Expression of microtubule-associated protein 2 by reactive astrocytes. *Proceedings of the National Academy of Sciences of the United States of America* 87(10), 3967-3971. doi: 10.1073/pnas.87.10.3967.

Gerges, N.Z., Backos, D.S., Rupasinghe, C.N., Spaller, M.R., and Esteban, J.A. (2006). Dual role of the exocyst in AMPA receptor targeting and insertion into the postsynaptic membrane. *Embo j* 25(8), 1623-1634. doi: 10.1038/sj.emboj.7601065.

Giese, K.P., and Mizuno, K. (2013). The roles of protein kinases in learning and memory. *Learn Mem* 20(10), 540-552. doi: 10.1101/lm.028449.112.

Giese, K.P., Fedorov, N.B., Filipkowski, R.K., and Silva, A.J. (1998). Autophosphorylation at Thr286 of the alpha calcium-calmodulin kinase II in LTP and learning. *Science* 279(5352), 870-873.

Gilbride, C.J. (2016). The hyperexcitability of dentate granule neurons in organotypic hippocampal slice cultures is due to reorganization of synaptic inputs in vitro. Physiological Reports 4(19), e12889. doi: 10.14814/phy2.12889.

Girard, L.R., Fiedler, T.J., Harris, T.W., Carvalho, F., Antoshechkin, I., Han, M., et al. (2007). WormBook: the online review of Caenorhabditis elegans biology. Nucleic Acids Res 35(Database issue), D472-475. doi: 10.1093/nar/gkl894.

Gisabella, B., Rowan, M.J., and Anwyl, R. (2003). Mechanisms underlying the inhibition of long-term potentiation by preconditioning stimulation in the hippocampus in vitro. *Neuroscience* 121(2), 297-305. doi: 10.1016/s0306-4522(03)00440-8.

Goel, A., Jiang, B., Xu, L.W., Song, L., Kirkwood, A., and Lee, H.K. (2006). Cross-modal regulation of synaptic AMPA receptors in primary sensory cortices by visual experience. *Nat Neurosci* 9(8), 1001-1003. doi: 10.1038/nn1725.

Golgi, C. (1885). *Sulla fina anatomia degli organi centrali del sistema nervoso.* S. Calderini.

Gomes, A.R., Correia, S.S., Esteban, J.A., Duarte, C.B., and Carvalho, A.L. (2007). PKC Anchoring to GluR4 AMPA Receptor Subunit Modulates PKC-Driven Receptor Phosphorylation and Surface Expression. *Traffic* 8(3), 259-269. doi: 10.1111/j.1600-0854.2006.00521.x.

Good, C.H., and Lupica, C.R. (2010). Afferent-specific AMPA receptor subunit composition and regulation of synaptic plasticity in midbrain dopamine neurons by abused drugs. *J Neurosci* 30(23), 7900-7909. doi: 10.1523/jneurosci.1507-10.2010.

Goodman, Y., and Mattson, M.P. (1994). Secreted forms of beta-amyloid precursor protein protect hippocampal neurons against amyloid beta-peptide-induced oxidative injury. *Exp Neurol* 128(1), 1-12. doi: 10.1006/exnr.1994.1107.

Goto, Y., and Grace, A.A. (2005). Dopamine-dependent interactions between limbic and prefrontal cortical plasticity in the nucleus accumbens: disruption by cocaine sensitization. *Neuron* 47(2), 255-266. doi: 10.1016/j.neuron.2005.06.017.

Gottschalk, W., Pozzo-Miller, L.D., Figurov, A., and Lu, B. (1998). Presynaptic modulation of synaptic transmission and plasticity by brain-derived neurotrophic factor in the developing hippocampus. *J Neurosci* 18(17), 6830-6839.

Götz, T., Kraushaar, U., Geiger, J., Lübke, J., Berger, T., and Jonas, P. (1997). Functional properties of AMPA and NMDA receptors expressed in identified types of basal ganglia neurons. *Journal of Neuroscience* 17(1), 204-215.

Grabrucker, A., Vaida, B., Bockmann, J., and Boeckers, T.M. (2009). Synaptogenesis of hippocampal neurons in primary cell culture. *Cell Tissue Res* 338(3), 333-341. doi: 10.1007/s00441-009-0881-z.

Gralle, M., Botelho, M.G., and Wouters, F.S. (2009). Neuroprotective secreted amyloid precursor protein acts by disrupting amyloid precursor protein dimers. *J Biol Chem* 284(22), 15016-15025. doi: 10.1074/jbc.M808755200.

Granado, N., Ortiz, O., Suárez, L.M., Martín, E.D., Ceña, V., Solís, J.M., et al. (2008). D1 but not D5 dopamine receptors are critical for LTP, spatial learning, and LTP-Induced arc and zif268 expression in the hippocampus. *Cereb Cortex* 18(1), 1-12. doi: 10.1093/cercor/bhm026.

Granger, A.J., Shi, Y., Lu, W., Cerpas, M., and Nicoll, R.A. (2013). LTP requires a reserve pool of glutamate receptors independent of subunit type. *Nature* 493(7433), 495-500. doi: 10.1038/nature11775.

Grassi, F., Palma, E., Tonini, R., Amici, M., Ballivet, M., and Eusebi, F. (2003). Amyloid beta(1-42) peptide alters the gating of human and mouse alpha-bungarotoxin-sensitive nicotinic receptors. *J Physiol* 547(Pt 1), 147-157. doi: 10.1113/jphysiol.2002.035436.

Gray, E.E., Fink, A.E., Sarinana, J., Vissel, B., and O'Dell, T.J. (2007). Long-term potentiation in the hippocampal CA1 region does not require insertion and activation of GluR2-lacking AMPA receptors. *J Neurophysiol* 98(4), 2488-2492. doi: 10.1152/jn.00473.2007.

Gray, R., Rajan, A.S., Radcliffe, K.A., Yakehiro, M., and Dani, J.A. (1996). Hippocampal synaptic transmission enhanced by low concentrations of nicotine. *Nature* 383(6602), 713-716. doi: 10.1038/383713a0.

Graziane, N.M., Sun, S., Wright, W.J., Jang, D., Liu, Z., Huang, Y.H., et al. (2016). Opposing mechanisms mediate morphine- and cocaine-induced generation of silent synapses. *Nat Neurosci* 19(7), 915-925. doi: 10.1038/nn.4313.

Greenberg, M.E., Xu, B., Lu, B., and Hempstead, B.L. (2009). New insights in the biology of BDNF synthesis and release: implications in CNS function. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 29(41), 12764-12767. doi: 10.1523/JNEUROSCI.3566-09.2009.

Greengard, P., Jen, J., Nairn, A.C., and Stevens, C.F. (1991). Enhancement of the glutamate response by cAMP-dependent protein kinase in hippocampal neurons. *Science* 253(5024), 1135-1138.

Greenough, W.T., and Volkmar, F.R. (1973). Pattern of dendritic branching in occipital cortex of rats reared in complex environments. *Experimental neurology* 40(2), 491-504.

Greger, I.H., Khatri, L., and Ziff, E.B. (2002). RNA editing at arg607 controls AMPA receptor exit from the endoplasmic reticulum. *Neuron* 34(5), 759-772. doi: 10.1016/s0896-6273(02)00693-1.

Greger, I.H., Khatri, L., Kong, X., and Ziff, E.B. (2003). AMPA Receptor Tetramerization Is Mediated by Q/R Editing. *Neuron* 40(4), 763-774. doi: https://doi.org/10.1016/S0896-6273(03)00668-8.

Gregor, A. (1907). Beiträge zur Kenntnis der Gedächtnisstörung bei der Korsakoffschen Psychose. *European Neurology* 21(1), 19-46.

Griffin, É.W., Mullally, S., Foley, C., Warmington, S.A., O'Mara, S.M., and Kelly, Á.M. (2011). Aerobic exercise improves hippocampal function and increases BDNF in the serum of young adult males. *Physiology & Behavior* 104(5), 934-941. doi: https://doi.org/10.1016/j.physbeh.2011.06.005.

Grinevich, V., Kolleker, A., Eliava, M., Takada, N., Takuma, H., Fukazawa, Y., et al. (2009). Fluorescent Arc/Arg3.1 indicator mice: a versatile tool to study brain activity changes in vitro and in vivo. *Journal of neuroscience methods* 184(1), 25-36. doi: 10.1016/j.jneumeth.2009.07.015.

Grintsevich, E.E., and Reisler, E. (2014). Drebrin inhibits cofilin-induced severing of F-actin. *Cytoskeleton* 71(8), 472-483. doi: 10.1002/cm.21184.

Grooms, S.Y., Noh, K.-M., Regis, R., Bassell, G.J., Bryan, M.K., Carroll, R.C., et al. (2006). Activity Bidirectionally Regulates AMPA Receptor mRNA Abundance in Dendrites of Hippocampal Neurons. *The Journal of Neuroscience* 26(32), 8339-8351. doi: 10.1523/jneurosci.0472-06.2006.

Grosskreutz, J., Zoerner, A., Schlesinger, F., Krampfl, K., Dengler, R., and Bufler, J. (2003). Kinetic properties of human AMPA-type glutamate receptors expressed in HEK293 cells. *Eur J Neurosci* 17(6), 1173-1178. doi: 10.1046/j.1460-9568.2003.02531.x.

Grünthal, E. (1947). Über das klinische Bild nach umschriebenem beiderseitigem Ausfall der Ammonshornrinde. *European Neurology* 113(1), 1-16.

Grybko, M., Sharma, G., and Vijayaraghavan, S. (2010). Functional distribution of nicotinic receptors in CA3 region of the hippocampus. *Journal of molecular neuroscience : MN* 40(1-2), 114-120. doi: 10.1007/s12031-009-9266-8.

Gu, J., Lee, C.W., Fan, Y., Komlos, D., Tang, X., Sun, C., et al. (2010). ADF/cofilin-mediated actin dynamics regulate AMPA receptor trafficking during synaptic plasticity. *Nature neuroscience* 13(10), 1208-1215. doi: 10.1038/nn.2634.

Gu, J., Tian, X., Wang, W., Yang, Q., Lin, P., Ma, Y., et al. (2018). Inhibition of Cgkii Suppresses Seizure Activity and Hippocampal Excitation by Regulating the Postsynaptic Delivery of Glua1. *Cellular Physiology and Biochemistry* 46(1), 160-177. doi: 10.1159/000488419.

Gu, Z., Liu, W., and Yan, Z. (2009). β-Amyloid Impairs AMPA Receptor Trafficking and Function by Reducing Ca^{2+}/Calmodulin-dependent Protein Kinase II Synaptic Distribution. *Journal of Biological Chemistry* 284(16), 10639-10649. doi: 10.1074/jbc.M806508200.

Gubbins, E.J., Gopalakrishnan, M., and Li, J. (2010). α7 nAChR-mediated activation of MAP kinase pathways in PC12 cells. *Brain Research* 1328, 1-11. doi: https://doi.org/10.1016/j.brainres.2010.02.083.

Gudi, T., Hong, G.K., Vaandrager, A.B., Lohmann, S.M., and Pilz, R.B. (1999). Nitric oxide and cGMP regulate gene expression in neuronal and glial cells by activating type II cGMP-dependent protein kinase. *Faseb j* 13(15), 2143-2152.

Guire, E.S., Oh, M.C., Soderling, T.R., and Derkach, V.A. (2008). Recruitment of Calcium-permeable AMPA Receptors During Synaptic Potentiation is Regulated by CaM-Kinase I. *J Neurosci* 28(23), 6000-6009. doi: 10.1523/jneurosci.0384-08.2008.

Gulisano, W., Melone, M., Ripoli, C., Tropea, M.R., Li Puma, D.D., Giunta, S., et al. (2019). Neuromodulatory Action of Picomolar Extracellular Aβ42 Oligomers on Presynaptic and Postsynaptic Mechanisms Underlying Synaptic Function and Memory. *The Journal of Neuroscience* 39(30), 5986-6000. doi: 10.1523/jneurosci.0163-19.2019.

Gumy, L.F., Katrukha, E.A., Grigoriev, I., Jaarsma, D., Kapitein, L.C., Akhmanova, A., et al. (2017). MAP2 Defines a Pre-axonal Filtering Zone to Regulate KIF1- versus KIF5-Dependent Cargo Transport in Sensory Neurons. *Neuron* 94(2), 347-362.e347. doi: https://doi.org/10.1016/j.neuron.2017.03.046.

Gunhanlar, N., Shpak, G., van der Kroeg, M., Gouty-Colomer, L.A., Munshi, S.T., Lendemeijer, B., et al. (2018). A simplified protocol for differentiation of electrophysiologically mature neuronal networks from human induced pluripotent stem cells. *Molecular Psychiatry* 23(5), 1336-1344. doi: 10.1038/mp.2017.56.

Guntupalli, S., Jang, S.E., Zhu, T., Huganir, R.L., Widagdo, J., and Anggono, V. (2017). GluA1 subunit ubiquitination mediates amyloid-β-induced loss of surface α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. *The Journal of biological chemistry* 292(20), 8186-8194. doi: 10.1074/jbc.M116.774554.

Guo, Y., Huang, S., de Pasquale, R., McGehrin, K., Lee, H.-K., Zhao, K., et al. (2012). Dark Exposure Extends the Integration Window for Spike-Timing-Dependent Plasticity. *The Journal of Neuroscience* 32(43), 15027-15035. doi: 10.1523/jneurosci.2545-12.2012.

Gupta, V., and Sharma, B. (2017). Role of phytochemicals in neurotrophins mediated regulation of Alzheimer's disease. *Int J Complement Alt Med* 7(4), 00231.

Guzowski, J.F., Lyford, G.L., Stevenson, G.D., Houston, F.P., McGaugh, J.L., Worley, P.F., et al. (2000). Inhibition of activity-dependent arc protein expression in the rat hippocampus impairs the maintenance of long-term potentiation and the consolidation of long-term memory. *J Neurosci* 20(11), 3993-4001.

Ha, D., Yang, N., and Nadithe, V. (2016). Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. *Acta Pharmaceutica Sinica B* 6(4), 287-296. doi: https://doi.org/10.1016/j.apsb.2016.02.001.

Haber, W.B. (1955). Effects of loss of limb on sensory functions. *The Journal of Psychology* 40(1), 115-123.

Haber, W.B. (1958). Reactions to loss of limb: physiological and psychological aspects. *Annals of the New York Academy of Sciences* 74(1), 14-24.

Haddad, J.J. (2005). N-methyl-D-aspartate (NMDA) and the regulation of mitogen-activated protein kinase (MAPK) signaling pathways: a revolving neurochemical axis for therapeutic intervention? *Prog Neurobiol* 77(4), 252-282. doi: 10.1016/j.pneurobio.2005.10.008.

Haj-Dahmane, S., Béïque, J.C., and Shen, R.-Y. (2017). GluA2-Lacking AMPA Receptors and Nitric Oxide Signaling Gate Spike-Timing-Dependent Potentiation of Glutamate Synapses in the Dorsal Raphe Nucleus. *eNeuro* 4(3), ENEURO.0116-0117.2017. doi: 10.1523/ENEURO.0116-17.2017.

Hall, J., Thomas, K.L., and Everitt, B.J. (2001). Cellular imaging of zif268 expression in the hippocampus and amygdala during contextual and cued fear memory retrieval: selective activation of hippocampal CA1 neurons during the recall of contextual memories. *J Neurosci* 21(6), 2186-2193.

Hammond, J.C., McCullumsmith, R.E., Funk, A.J., Haroutunian, V., and Meador-Woodruff, J.H. (2010). Evidence for abnormal forward trafficking of AMPA receptors in frontal cortex of elderly patients with schizophrenia. *Neuropsychopharmacology* 35(10), 2110-2119. doi: 10.1038/npp.2010.87.

Haney, M.J., Klyachko, N.L., Zhao, Y., Gupta, R., Plotnikova, E.G., He, Z., et al. (2015). Exosomes as drug delivery vehicles for Parkinson's disease therapy. *Journal of controlled release : official journal of the Controlled Release Society* 207, 18-30. doi: 10.1016/j.jconrel.2015.03.033.

Hangen, E., Cordelières, F.P., Petersen, J.D., Choquet, D., and Coussen, F. (2018). Neuronal Activity and Intracellular Calcium Levels Regulate Intracellular Transport of Newly Synthesized AMPAR. *Cell Reports* 24(4), 1001-1012.e1003. doi: https://doi.org/10.1016/j.celrep.2018.06.095.

Hanley, J.G. (2007). NSF binds calcium to regulate its interaction with AMPA receptor subunit GluR2. *Journal of Neurochemistry* 101(6), 1644-1650. doi: 10.1111/j.1471-4159.2007.04455.x.

Hanley, J.G. (2014). Actin-dependent mechanisms in AMPA receptor trafficking. *Frontiers in cellular neuroscience* 8, 381-381. doi: 10.3389/fncel.2014.00381.

Hanley, J.G. (2018). The Regulation of AMPA Receptor Endocytosis by Dynamic Protein-Protein Interactions. *Frontiers in Cellular Neuroscience* 12(362). doi: 10.3389/fncel.2018.00362.

Hanley, J.G., Khatri, L., Hanson, P.I., and Ziff, E.B. (2002). NSF ATPase and alpha-/beta-SNAPs disassemble the AMPA receptor-PICK1 complex. *Neuron* 34(1), 53-67. doi: 10.1016/s0896-6273(02)00638-4.

Hardingham, N., and Fox, K. (2006). The Role of Nitric Oxide and GluR1 in Presynaptic and Postsynaptic Components of Neocortical Potentiation. *The Journal of Neuroscience* 26(28), 7395-7404. doi: 10.1523/jneurosci.0652-06.2006.

Hardt, O., Nader, K., and Wang, Y.-T. (2013). GluA2-dependent AMPA receptor endocytosis and the decay of early and late long-term potentiation: possible mechanisms for forgetting of short- and long-term memories. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 369(1633), 20130141-20130141. doi: 10.1098/rstb.2013.0141.

Harms, K.J., Tovar, K.R., and Craig, A.M. (2005). Synapse-Specific Regulation of AMPA Receptor Subunit Composition by Activity. *The Journal of Neuroscience* 25(27), 6379-6388. doi: 10.1523/jneurosci.0302-05.2005.

Harrill, J.A., Chen, H., Streifel, K.M., Yang, D., Mundy, W.R., and Lein, P.J. (2015). Ontogeny of biochemical, morphological and functional parameters of synaptogenesis in primary cultures of rat hippocampal and cortical neurons. *Molecular brain* 8, 10-10. doi: 10.1186/s13041-015-0099-9.

Harris, E.W., and Cotman, C.W. (1986). Long-term potentiation of guinea pig mossy fiber responses is not blocked by N-methyl D-aspartate antagonists. *Neurosci Lett* 70(1), 132-137. doi: 10.1016/0304-3940(86)90451-9.

Harrison, P.J., McLaughlin, D., and Kerwin, R.W. (1991). Decreased hippocampal expression of a glutamate receptor gene in schizophrenia. *Lancet* 337(8739), 450-452. doi: 10.1016/0140-6736(91)93392-m.

Hartl, D., Klatt, S., Roch, M., Konthur, Z., Klose, J., Willnow, T.E., et al. (2013). Soluble Alpha-APP (sAPPalpha) Regulates CDK5 Expression and Activity in Neurons. *PLOS ONE* 8(6), e65920. doi: 10.1371/journal.pone.0065920.

Hartman, M.A., Finan, D., Sivaramakrishnan, S., and Spudich, J.A. (2011). Principles of unconventional myosin function and targeting. *Annu Rev Cell Dev Biol* 27, 133-155. doi: 10.1146/annurev-cellbio-100809-151502.

Haucke, V., Neher, E., and Sigrist, S.J. (2011). Protein scaffolds in the coupling of synaptic exocytosis and endocytosis. *Nature Reviews Neuroscience* 12(3), 127-138. doi: 10.1038/nrn2948.

Hawkes, N. (2017). Merck ends trial of potential Alzheimer's drug verubecestat. *Bmj* 356, j845. doi: 10.1136/bmj.j845.

Hayashi, K., and Shirao, T. (1999). Change in the Shape of Dendritic Spines Caused by Overexpression of Drebrin in Cultured Cortical Neurons. *The Journal of Neuroscience* 19(10), 3918-3925. doi: 10.1523/jneurosci.19-10-03918.1999.

Hayashi, Y., Nishio, M., Naito, Y., Yokokura, H., Nimura, Y., Hidaka, H., et al. (1999). Regulation of neuronal nitric-oxide synthase by calmodulin kinases. *J Biol Chem* 274(29), 20597-20602. doi: 10.1074/jbc.274.29.20597.

Hayashi, Y., Shi, S.-H., Esteban, J.A., Piccini, A., Poncer, J.-C., and Malinow, R. (2000). Driving AMPA Receptors into Synapses by LTP and CaMKII: Requirement for GluR1 and PDZ Domain Interaction. *Science* 287(5461), 2262-2267. doi: 10.1126/science.287.5461.2262.

Haydon, P.G., and Carmignoto, G. (2006). Astrocyte control of synaptic transmission and neurovascular coupling. *Physiol Rev* 86(3), 1009-1031. doi: 10.1152/physrev.00049.2005.

He, K., Song, L., Cummings, L.W., Goldman, J., Huganir, R.L., and Lee, H.K. (2009). Stabilization of Ca^{2+}-permeable AMPA receptors at perisynaptic sites by GluR1-S845 phosphorylation. *Proc Natl Acad Sci U S A* 106(47), 20033-20038. doi: 10.1073/pnas.0910338106.

Hebb, D.O. (1949). The organization of behavior a neuropsychological theory.

Hebert, D.N., and Molinari, M. (2007). In and out of the ER: protein folding, quality control, degradation, and related human diseases. *Physiol Rev* 87(4), 1377-1408. doi: 10.1152/physrev.00050.2006.

Hébert, S.S., Serneels, L., Dejaegere, T., Horré, K., Dabrowski, M., Baert, V., et al. (2004). Coordinated and widespread expression of γ-secretase in vivo: evidence for size and molecular heterogeneity. *Neurobiology of Disease* 17(2), 260-272. doi: https://doi.org/10.1016/j.nbd.2004.08.002.

Hefferan MP, Kucharova K, Kinjo K, Kakinohana O, Sekerkova G, Nakamura S, Fuchigami T, Tomori Z, Yaksh TL, Kurtz N, Marsala M (2007) Spinal Astrocyte Glutamate Receptor 1 Overexpression after Ischemic Insult Facilitates Behavioral Signs of Spasticity and Rigidity. The Journal of Neuroscience 27:11179-11191.

Hefft S, Hulo S, Bertrand D, Muller D (1999) Synaptic transmission at nicotinic acetylcholine receptors in rat hippocampal organotypic cultures and slices. The Journal of physiology 515 (Pt 3):769-776.

Hefter, D., Kaiser, M., Weyer, S.W., Papageorgiou, I.E., Both, M., Kann, O., et al. (2016). Amyloid Precursor Protein Protects Neuronal Network Function after Hypoxia via Control of Voltage-Gated Calcium Channels. *J Neurosci* 36(32), 8356-8371. doi: 10.1523/jneurosci.4130-15.2016.

Heinrichs, S.C., Leite-Morris, K.A., Guy, M.D., Goldberg, L.R., Young, A.J., and Kaplan, G.B. (2013). Dendritic structural plasticity in the basolateral amygdala after fear conditioning and its extinction in mice. *Behavioural brain research* 248, 80-84. doi: 10.1016/j.bbr.2013.03.048.

Hell, J.W. (2016). How Ca²⁺-permeable AMPA receptors, the kinase PKA, and the phosphatase PP2B are intertwined in synaptic LTP and LTD. *Science Signaling* 9(425), pe2-pe2. doi: 10.1126/scisignal.aaf7067.

Helmuth, L. (2001). Neuroscience. Glia tell neurons to build synapses. *Science* 291(5504), 569-570. doi: 10.1126/science.291.5504.569a.

Henley, J.M. (2003). Proteins interactions implicated in AMPA receptor trafficking: a clear destination and an improving route map. *Neuroscience research* 45(3), 243-254. doi: 10.1016/s0168-0102(02)00229-8.

Henley, J.M., and Wilkinson, K.A. (2013). AMPA receptor trafficking and the mechanisms underlying synaptic plasticity and cognitive aging. *Dialogues in clinical neuroscience* 15(1), 11-27.

Henley, J.M., and Wilkinson, K.A. (2016). Synaptic AMPA receptor composition in development, plasticity and disease. *Nat Rev Neurosci* 17(6), 337-350. doi: 10.1038/nrn.2016.37.

Hennig, M. (2013). Theoretical models of synaptic short term plasticity. *Frontiers in Computational Neuroscience* 7(45). doi: 10.3389/fncom.2013.00045.

Henry, A., Li, Q.X., Galatis, D., Hesse, L., Multhaup, G., Beyreuther, K., et al. (1998). Inhibition of platelet activation by the Alzheimer's disease amyloid precursor protein. *Br J Haematol* 103(2), 402-415. doi: 10.1046/j.1365-2141.1998.01005.x.

Henson, M.A., Tucker, C.J., Zhao, M., and Dudek, S.M. (2017). Long-term depression-associated signaling is required for an in vitro model of NMDA receptor-dependent synapse pruning. *Neurobiology of Learning and Memory* 138, 39-53. doi: https://doi.org/10.1016/j.nlm.2016.10.013.

Herguedas, B., Krieger, J., and Greger, I.H. (2013). "Chapter Thirteen - Receptor Heteromeric Assembly—How It Works and Why It Matters: The Case of Ionotropic Glutamate Receptors," in *Progress in Molecular Biology and Translational Science,* eds. J. Giraldo & F. Ciruela. Academic Press), 361-386.

Herguedas, B., Watson, J.F., Ho, H., Cais, O., García-Nafría, J., and Greger, I.H. (2019). Architecture of the heteromeric GluA1/2 AMPA receptor in complex with the auxiliary subunit TARP γ8. *Science*, eaav9011. doi: 10.1126/science.aav9011.

Herms, J., Anliker, B., Heber, S., Ring, S., Fuhrmann, M., Kretzschmar, H., et al. (2004). Cortical dysplasia resembling human type 2 lissencephaly in mice lacking all three APP family members. *Embo j* 23(20), 4106-4115. doi: 10.1038/sj.emboj.7600390.

Herring, B.E., Shi, Y., Suh, Y.H., Zheng, C.-Y., Blankenship, S.M., Roche, K.W., et al. (2013). Cornichon proteins determine the subunit composition of synaptic AMPA receptors. *Neuron* 77(6), 1083-1096. doi: 10.1016/j.neuron.2013.01.017.

Hesse, R., Lausser, L., Gummert, P., Schmid, F., Wahler, A., Schnack, C., et al. (2017). Reduced cGMP levels in CSF of AD patients correlate with severity of dementia and current depression. *Alzheimer's Research & Therapy* 9(1), 17. doi: 10.1186/s13195-017-0245-y.

Hick, M., Herrmann, U., Weyer, S.W., Mallm, J.P., Tschape, J.A., Borgers, M., et al. (2015). Acute function of secreted amyloid precursor protein fragment APPsalpha in synaptic plasticity. *Acta Neuropathol* 129(1), 21-37. doi: 10.1007/s00401-014-1368-x.

Ho, M.T., Pelkey, K.A., Topolnik, L., Petralia, R.S., Takamiya, K., Xia, J., et al. (2007). Developmental expression of Ca^{2+}-permeable AMPA receptors underlies depolarization-induced long-term depression at mossy fiber CA3 pyramid synapses. *J Neurosci* 27(43), 11651-11662. doi: 10.1523/jneurosci.2671-07.2007.

Hock, C., Golombowski, S., Muller-Spahn, F., Naser, W., Beyreuther, K., Monning, U., et al. (1998). Cerebrospinal fluid levels of amyloid precursor protein and amyloid beta-peptide in Alzheimer's disease and major depression - inverse correlation with dementia severity. *Eur Neurol* 39(2), 111-118. doi: 10.1159/000007917.

Hoe, H.-S., Fu, Z., Makarova, A., Lee, J.-Y., Lu, C., Feng, L., et al. (2009). The Effects of Amyloid Precursor Protein on Postsynaptic Composition and Activity. *Journal of Biological Chemistry* 284(13), 8495-8506. doi: 10.1074/jbc.M900141200.

Hoerndli, F.J., Maxfield, D.A., Brockie, P.J., Mellem, J.E., Jensen, E., Wang, R., et al. (2013). Kinesin-1 regulates synaptic strength by mediating the delivery, removal, and redistribution of AMPA receptors. *Neuron* 80(6), 1421-1437. doi: 10.1016/j.neuron.2013.10.050.

Hoerndli, F.J., Wang, R., Mellem, J.E., Kallarackal, A., Brockie, P.J., Thacker, C., et al. (2015). Neuronal Activity and CaMKII Regulate Kinesin-Mediated Transport of Synaptic AMPARs. *Neuron* 86(2), 457-474. doi: 10.1016/j.neuron.2015.03.011.

Hofer, M.M., and Barde, Y.A. (1988). Brain-derived neurotrophic factor prevents neuronal death in vivo. *Nature* 331(6153), 261-262. doi: 10.1038/331261a0.

Holman, D., Feligioni, M., and Henley, J.M. (2007). Differential redistribution of native AMPA receptor complexes following LTD induction in acute hippocampal slices. *Neuropharmacology* 52(1), 92-99. doi: 10.1016/j.neuropharm.2006.05.022.

Hong, I., Kim, J., Kim, J., Lee, S., Ko, H.-G., Nader, K., et al. (2013). AMPA receptor exchange underlies transient memory destabilization on retrieval. *Proceedings of the National Academy of Sciences* 110(20), 8218-8223. doi: 10.1073/pnas.1305235110.

Hong, I., Kim, J., Kim, J., Lee, S., Ko, H.-G., Nader, K., et al. (2013). AMPA receptor exchange underlies transient memory destabilization on retrieval. *Proceedings of the National Academy of Sciences* 110(20), 8218-8223. doi: 10.1073/pnas.1305235110.

Hongpaisan, J., Xu, C., Sen, A., Nelson, T.J., and Alkon, D.L. (2013). PKC activation during training restores mushroom spine synapses and memory in the aged rat. *Neurobiol Dis* 55, 44-62. doi: 10.1016/j.nbd.2013.03.012.

Horizon Discovery. (2014). *Dharmacon™ RNAi & Gene Expression Product Guide*. Retrieved from https://www.cytivalifesciences.co.jp/technologies/Dharmacon_productGuide_2014_sml-spread.pdf

Horsch, M., Seeburg, P.H., Adler, T., Aguilar-Pimentel, J.A., Becker, L., Calzada-Wack, J., et al. (2011). Requirement of the RNA-editing enzyme ADAR2 for normal physiology in mice. *J Biol Chem* 286(21), 18614-18622. doi: 10.1074/jbc.M110.200881.

Horton, A.C., and Ehlers, M.D. (2003). Dual modes of endoplasmic reticulum-to-Golgi transport in dendrites revealed by live-cell imaging. *J Neurosci* 23(15), 6188-6199. doi: 10.1523/jneurosci.23-15-06188.2003.

Horton, A.C., Rácz, B., Monson, E.E., Lin, A.L., Weinberg, R.J., and Ehlers, M.D. (2005). Polarized secretory trafficking directs cargo for asymmetric dendrite growth and morphogenesis. *Neuron* 48(5), 757-771. doi: 10.1016/j.neuron.2005.11.005.

Hosoi, N., Holt, M., and Sakaba, T. (2009). Calcium dependence of exo- and endocytotic coupling at a glutamatergic synapse. *Neuron* 63(2), 216-229. doi: 10.1016/j.neuron.2009.06.010.

Hossaini, M., Jongen, J.L.M., Biesheuvel, K., Kuhl, D., and Holstege, J.C. (2010). Nociceptive stimulation induces expression of Arc/Arg3.1 in the spinal cord with a preference for neurons containing enkephalin. *Molecular pain* 6, 43-43. doi: 10.1186/1744-8069-6-43.

Hou, Q., Gilbert, J., and Man, H.-Y. (2011). Homeostatic regulation of AMPA receptor trafficking and degradation by light-controlled single synaptic activation. *Neuron* 72(5), 806-818. doi: 10.1016/j.neuron.2011.10.011.

Hou, Q., Zhang, D., Jarzylo, L., Huganir, R.L., and Man, H.-Y. (2008). Homeostatic regulation of AMPA receptor expression at single hippocampal synapses. *Proceedings of the National Academy of Sciences* 105(2), 775-780. doi: 10.1073/pnas.0706447105.

Howarth, M., Takao, K., Hayashi, Y., and Ting, A.Y. (2005). Targeting quantum dots to surface proteins in living cells with biotin ligase. *Proc Natl Acad Sci U S A* 102(21), 7583-7588. doi: 10.1073/pnas.0503125102.

Hsia, A.Y., Masliah, E., McConlogue, L., Yu, G.Q., Tatsuno, G., Hu, K., et al. (1999). Plaque-independent disruption of neural circuits in Alzheimer's disease mouse models. *Proc Natl Acad Sci U S A* 96(6), 3228-3233. doi: 10.1073/pnas.96.6.3228.

Hsieh, H., Boehm, J., Sato, C., Iwatsubo, T., Tomita, T., Sisodia, S., et al. (2006). AMPAR removal underlies Abeta-induced synaptic depression and dendritic spine loss. *Neuron* 52(5), 831-843. doi: 10.1016/j.neuron.2006.10.035.

Hu, G.Y., Hvalby, O., Walaas, S.I., Albert, K.A., Skjeflo, P., Andersen, P., et al. (1987). Protein kinase C injection into hippocampal pyramidal cells elicits features of long term potentiation. *Nature* 328(6129), 426-429. doi: 10.1038/328426a0.

Hu, J.-Y., Glickman, L., Wu, F., and Schacher, S. (2004). Serotonin Regulates the Secretion and Autocrine Action of a Neuropeptide to Activate MAPK Required for Long-Term Facilitation in Aplysia. *Neuron* 43(3), 373-385. doi: https://doi.org/10.1016/j.neuron.2004.07.011.

Huang, B., Bates, M., and Zhuang, X. (2009). Super-resolution fluorescence microscopy. *Annual review of biochemistry* 78, 993-1016. doi: 10.1146/annurev.biochem.77.061906.092014.

Huang, C.C., and Hsu, K.S. (2001). Progress in understanding the factors regulating reversibility of long-term potentiation. *Rev Neurosci* 12(1), 51-68.

Huang, F., Chotiner, J.K., and Steward, O. (2007). Actin polymerization and ERK phosphorylation are required for Arc/Arg3.1 mRNA targeting to activated synaptic sites on dendrites. *J Neurosci* 27(34), 9054-9067. doi: 10.1523/jneurosci.2410-07.2007.

Huang, Y.-Y., and Kandel, E.R. (1995). D1/D5 receptor agonists induce a protein synthesis-dependent late potentiation in the CA1 region of the hippocampus. *Proceedings of the National Academy of Sciences* 92(7), 2446-2450.

Huang, Y.-Y., and Kandel, E.R. (2005). Theta frequency stimulation induces a local form of late phase LTP in the CA1 region of the hippocampus. *Learning & Memory* 12(6), 587-593. doi: 10.1101/lm.98905.

Huang, Y.H., Lin, Y., Mu, P., Lee, B.R., Brown, T.E., Wayman, G., et al. (2009). In vivo cocaine experience generates silent synapses. *Neuron* 63(1), 40-47. doi: 10.1016/j.neuron.2009.06.007.

Huang, Y.S., Kan, M.C., Lin, C.L., and Richter, J.D. (2006). CPEB3 and CPEB4 in neurons: analysis of RNA-binding specificity and translational control of AMPA receptor GluR2 mRNA. *Embo j* 25(20), 4865-4876. doi: 10.1038/sj.emboj.7601322.

Huang, Y.Y., and Kandel, E.R. (1994). Recruitment of long-lasting and protein kinase A-dependent long-term potentiation in the CA1 region of hippocampus requires repeated tetanization. *Learn Mem* 1(1), 74-82.

Huang, Y.Y., Colino, A., Selig, D.K., and Malenka, R.C. (1992). The influence of prior synaptic activity on the induction of long-term potentiation. *Science* 255(5045), 730-733. doi: 10.1126/science.1346729.

Huang, Y.Y., Kandel, D.B., Kandel, E.R., and Levine, A. (2013). Nicotine primes the effect of cocaine on the induction of LTP in the amygdala. *Neuropharmacology* 74, 126-134. doi: 10.1016/j.neuropharm.2013.03.031.

Huber, K.M., Kayser, M.S., and Bear, M.F. (2000). Role for Rapid Dendritic Protein Synthesis in Hippocampal mGluR-Dependent Long-Term Depression. *Science* 288(5469), 1254-1256. doi: 10.1126/science.288.5469.1254.

Hudgins, C., and Otto, T. (2019). Hippocampal Arc protein expression and conditioned fear. *Neurobiology of Learning and Memory* 161, 175-191. doi: https://doi.org/10.1016/j.nlm.2019.04.004.

Humeau, Y., Reisel, D., Johnson, A.W., Borchardt, T., Jensen, V., Gebhardt, C., et al. (2007). A pathway-specific function for different AMPA receptor subunits in amygdala long-term potentiation and fear conditioning. *J Neurosci* 27(41), 10947-10956. doi: 10.1523/jneurosci.2603-07.2007.

Hung, A.Y., Haass, C., Nitsch, R.M., Qiu, W.Q., Citron, M., Wurtman, R.J., et al. (1993). Activation of protein kinase C inhibits cellular production of the amyloid beta-protein. *J Biol Chem* 268(31), 22959-22962.

Huupponen, J., Atanasova, T., Taira, T., and Lauri, S.E. (2016). GluA4 subunit of AMPA receptors mediates the early synaptic response to altered network activity in the developing hippocampus. *Journal of neurophysiology* 115(6), 2989-2996. doi: 10.1152/jn.00435.2015.

Iacobucci, G.J., and Popescu, G.K. (2017). NMDA receptors: linking physiological output to biophysical operation. *Nat Rev Neurosci* 18(4), 236-249. doi: 10.1038/nrn.2017.24.

Impey, S., Smith, D.M., Obrietan, K., Donahue, R., Wade, C., and Storm, D.R. (1998). Stimulation of cAMP response element (CRE)-mediated transcription during contextual learning. *Nat Neurosci* 1(7), 595-601. doi: 10.1038/2830.

Incontro, S., Ciruela, F., Ziff, E., Hofmann, F., Sánchez-Prieto, J., and Torres, M. (2013). The type II cGMP dependent protein kinase regulates GluA1 levels at the plasma membrane of developing cerebellar granule cells. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research* 1833(8), 1820-1831. doi: https://doi.org/10.1016/j.bbamcr.2013.03.021.

Innocent, N., Cousins, S.L., and Stephenson, F.A. (2012). NMDA receptor/amyloid precursor protein interactions: a comparison between wild-type and amyloid precursor protein mutations associated with familial Alzheimer's disease. *Neurosci Lett* 515(2), 131-136. doi: 10.1016/j.neulet.2012.03.029.

Inoue, S., Shikanai, H., Matsumoto, M., Hiraide, S., Saito, Y., Yanagawa, Y., et al. (2014). Metaplastic regulation of the median raphe nucleus via serotonin 5-HT1A receptor on hippocampal synaptic plasticity is associated with gender-specific emotional expression in rats. *J Pharmacol Sci* 124(3), 394-407. doi: 10.1254/jphs.13237fp.

Inoue, W., Baimoukhametova, D.V., Füzesi, T., Wamsteeker Cusulin, J.I., Koblinger, K., Whelan, P.J., et al. (2013). Noradrenaline is a stress-associated metaplastic signal at GABA synapses. *Nature neuroscience* 16(5), 605-612. doi: 10.1038/nn.3373.

Insausti, R. (1993). Comparative anatomy of the entorhinal cortex and hippocampus in mammals. *Hippocampus* 3(S1), 19-26. doi: 10.1002/hipo.1993.4500030705.

Isaac, J.T., Nicoll, R.A., and Malenka, R.C. (1995). Evidence for silent synapses: implications for the expression of LTP. *Neuron* 15(2), 427-434. doi: 10.1016/0896-6273(95)90046-2.

Isaac, J.T.R., Ashby, M.C., and McBain, C.J. (2007). The Role of the GluR2 Subunit in AMPA Receptor Function and Synaptic Plasticity. *Neuron* 54(6), 859-871. doi: https://doi.org/10.1016/j.neuron.2007.06.001.

Isaacson, L.G., Saffran, B.N., and Crutcher, K.A. (1990). Intracerebral NGF infusion induces hyperinnervation of cerebral blood vessels. *Neurobiology of Aging* 11(1), 51-55. doi: https://doi.org/10.1016/0197-4580(90)90062-5.

Ishida, A., Furukawa, K., Keller, J.N., and Mattson, M.P. (1997). Secreted form of beta-amyloid precursor protein shifts the frequency dependency for induction of LTD, and enhances LTP in hippocampal slices. *Neuroreport* 8(9-10), 2133-2137. doi: 10.1097/00001756-199707070-00009.

Isiegas, C., Park, A., Kandel, E.R., Abel, T., and Lattal, K.M. (2006). Transgenic Inhibition of Neuronal Protein Kinase A Activity Facilitates Fear Extinction. *The Journal of Neuroscience* 26(49), 12700-12707. doi: 10.1523/jneurosci.2743-06.2006.

Ismailov, I., Kalikulov, D., Inoue, T., and Friedlander, M.J. (2004). The kinetic profile of intracellular calcium predicts long-term potentiation and long-term depression. *J Neurosci* 24(44), 9847-9861. doi: 10.1523/jneurosci.0738-04.2004.

Itami, C., Kimura, F., Kohno, T., Matsuoka, M., Ichikawa, M., Tsumoto, T., et al. (2003). Brain-derived neurotrophic factor-dependent unmasking of "silent" synapses in the developing mouse barrel cortex. *Proc Natl Acad Sci U S A* 100(22), 13069-13074. doi: 10.1073/pnas.2131948100.

Ivanov, A., Esclapez, M., Pellegrino, C., Shirao, T., and Ferhat, L. (2009). Drebrin A regulates dendritic spine plasticity and synaptic function in mature cultured hippocampal neurons. *Journal of Cell Science* 122(4), 524-534. doi: 10.1242/jcs.033464.

Iwano, T., Masuda, A., Kiyonari, H., Enomoto, H., and Matsuzaki, F. (2012). Prox1 postmitotically defines dentate gyrus cells by specifying granule cell identity over CA3 pyramidal cell fate in the hippocampus. *Development* 139(16), 3051-3062. doi: 10.1242/dev.080002.

Izumi, Y., and Zorumski, C.F. (1999). Norepinephrine promotes long-term potentiation in the adult rat hippocampus in vitro. *Synapse* 31(3), 196-202. doi: 10.1002/(sici)1098-2396(19990301)31:3<196::Aid-syn4>3.0.Co;2-k.

Jaafari, N., Henley, J.M., and Hanley, J.G. (2012). PICK1 mediates transient synaptic expression of GluA2-lacking AMPA receptors during glycine-induced AMPA receptor trafficking. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 32(34), 11618-11630. doi: 10.1523/jneurosci.5068-11.2012.

Jabs, R., Kirchhoff, F., Kettenmann, H., and Steinhauser, C. (1994). Kainate activates Ca(2+)-permeable glutamate receptors and blocks voltage-gated K+ currents in glial cells of mouse hippocampal slices. *Pflugers Arch* 426(3-4), 310-319.

Jackman, S.L., Turecek, J., Belinsky, J.E., and Regehr, W.G. (2016). The calcium sensor synaptotagmin 7 is required for synaptic facilitation. *Nature* 529, 88. doi: 10.1038/nature16507.

Jacob, A.L., and Weinberg, R.J. (2015). The organization of AMPA receptor subunits at the postsynaptic membrane. *Hippocampus* 25(7), 798-812. doi: 10.1002/hipo.22404.

Jahr, C.E., and Stevens, C.F. (1993). Calcium permeability of the N-methyl-D-aspartate receptor channel in hippocampal neurons in culture. *Proc Natl Acad Sci U S A* 90(24), 11573-11577.

Jaken, S. (1996). Protein kinase C isozymes and substrates. *Current Opinion in Cell Biology* 8(2), 168-173. doi: https://doi.org/10.1016/S0955-0674(96)80062-7.

Jakkamsetti, V., Tsai, N.-P., Gross, C., Molinaro, G., Collins, Katie A., Nicoletti, F., et al. (2013a). Experience-Induced Arc/Arg3.1 Primes CA1 Pyramidal Neurons for Metabotropic Glutamate Receptor-Dependent Long-Term Synaptic Depression. *Neuron* 80(1), 72-79. doi: 10.1016/j.neuron.2013.07.020.

Jakkamsetti, V., Tsai, N.P., Gross, C., Molinaro, G., Collins, K.A., Nicoletti, F., et al. (2013b). Experience-induced Arc/Arg3.1 primes CA1 pyramidal neurons for metabotropic glutamate receptor-dependent long-term synaptic depression. *Neuron* 80(1), 72-79. doi: 10.1016/j.neuron.2013.07.020.

Jakobsson, J., Zetterberg, H., Blennow, K., Johan Ekman, C., Johansson, A.G., and Landen, M. (2013). Altered concentrations of amyloid precursor protein metabolites in the cerebrospinal fluid of patients with bipolar disorder. *Neuropsychopharmacology* 38(4), 664-672. doi: 10.1038/npp.2012.231.

Jalan-Sakrikar, N., Bartlett, R.K., Baucum, A.J., 2nd, and Colbran, R.J. (2012). Substrate-selective and calcium-independent activation of CaMKII by alpha-actinin. *J Biol Chem* 287(19), 15275-15283. doi: 10.1074/jbc.M112.351817.

James, W. (1890). *The Principles of Psychology: The Principles of Psychology.* Hansie old and expensive.

James, W. (2007). *The principles of psychology.* Cosimo, Inc.

Janelidze, S., Zetterberg, H., Mattsson, N., Palmqvist, S., Vanderstichele, H., Lindberg, O., et al. (2016). CSF Aβ42/Aβ40 and Aβ42/Aβ38 ratios: better diagnostic markers of Alzheimer disease. *Ann Clin Transl Neurol* 3(3), 154-165. doi: 10.1002/acn3.274.

Janz, P., Hauser, P., Heining, K., Nestel, S., Kirsch, M., Egert, U., et al. (2018). Position- and Time-Dependent Arc Expression Links Neuronal Activity to Synaptic Plasticity During Epileptogenesis. *Frontiers in Cellular Neuroscience* 12(244). doi: 10.3389/fncel.2018.00244.

Jarrett, J.T., Berger, E.P., and Lansbury, P.T., Jr. (1993). The carboxy terminus of the beta amyloid protein is critical for the seeding of amyloid formation: implications for the pathogenesis of Alzheimer's disease. *Biochemistry* 32(18), 4693-4697. doi: 10.1021/bi00069a001.

Jedlicka, P., Vlachos, A., Schwarzacher, S.W., and Deller, T. (2008). A role for the spine apparatus in LTP and spatial learning. *Behav Brain Res* 192(1), 12-19. doi: 10.1016/j.bbr.2008.02.033.

Jedlicka, P., Vnencak, M., Krueger, D.D., Jungenitz, T., Brose, N., and Schwarzacher, S.W. (2015). Neuroligin-1 regulates excitatory synaptic transmission, LTP and EPSP-spike coupling in the dentate gyrus in vivo. *Brain Struct Funct* 220(1), 47-58. doi: 10.1007/s00429-013-0636-1.

Jenkins, M.A., and Traynelis, S.F. (2012). PKC phosphorylates GluA1-Ser831 to enhance AMPA receptor conductance. *Channels (Austin)* 6(1), 60-64. doi: 10.4161/chan.18648.

Jenkins, M.A., Wells, G., Bachman, J., Snyder, J.P., Jenkins, A., Huganir, R.L., et al. (2014). Regulation of GluA1 alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor function by protein kinase C at serine-818 and threonine-840. *Mol Pharmacol* 85(4), 618-629. doi: 10.1124/mol.113.091488.

Jensen, V., Kaiser, K.M., Borchardt, T., Adelmann, G., Rozov, A., Burnashev, N., et al. (2003). A juvenile form of postsynaptic hippocampal long-term potentiation in mice deficient for the AMPA receptor subunit GluR-A. *J Physiol* 553(Pt 3), 843-856. doi: 10.1113/jphysiol.2003.053637.

Ji, Y., Lu, Y., Yang, F., Shen, W., Tang, T.T.-T., Feng, L., et al. (2010). Acute and gradual increases in BDNF concentration elicit distinct signaling and functions in neurons. *Nature Neuroscience* 13(3), 302-309. doi: 10.1038/nn.2505.

Jia, Z., Agopyan, N., Miu, P., Xiong, Z., Henderson, J., Gerlai, R., et al. (1996). Enhanced LTP in Mice Deficient in the AMPA Receptor GluR2. *Neuron* 17(5), 945-956. doi: 10.1016/S0896-6273(00)80225-1.

Jiang, J., Suppiramaniam, V., and Wooten, M.W. (2006). Posttranslational modifications and receptor-associated proteins in AMPA receptor trafficking and synaptic plasticity. *Neurosignals* 15(5), 266-282. doi: 10.1159/000105517.

Jin, L.W., Ninomiya, H., Roch, J.M., Schubert, D., Masliah, E., Otero, D.A., et al. (1994). Peptides containing the RERMS sequence of amyloid beta/A4 protein precursor bind cell surface and promote neurite extension. *J Neurosci* 14(9), 5461-5470.

Jitsuki, S., Takemoto, K., Kawasaki, T., Tada, H., Takahashi, A., Becamel, C., et al. (2011). Serotonin mediates cross-modal reorganization of cortical circuits. *Neuron* 69(4), 780-792. doi: 10.1016/j.neuron.2011.01.016.

John, D., Shelukhina, I., Yanagawa, Y., Deuchars, J., and Henderson, Z. (2015). Functional alpha7 nicotinic receptors are expressed on immature granule cells of the postnatal dentate gyrus. *Brain Research* 1601, 15-30. doi: https://doi.org/10.1016/j.brainres.2014.12.041.

Jourdi, H., and Kabbaj, M. (2013). Acute BDNF Treatment Upregulates GluR1-SAP97 and GluR2-GRIP1 Interactions: Implications for Sustained AMPA Receptor Expression. *PLOS ONE* 8(2), e57124. doi: 10.1371/journal.pone.0057124.

Ju, W., Morishita, W., Tsui, J., Gaietta, G., Deerinck, T.J., Adams, S.R., et al. (2004). Activity-dependent regulation of dendritic synthesis and trafficking of AMPA receptors. *Nat Neurosci* 7(3), 244-253. doi: 10.1038/nn1189.

Jungenitz, T., Beining, M., Radic, T., Deller, T., Cuntz, H., Jedlicka, P., et al. (2018). Structural homo- and heterosynaptic plasticity in mature and adult newborn rat hippocampal granule cells. *Proceedings of the National Academy of Sciences* 115(20), E4670-E4679. doi: 10.1073/pnas.1801889115.

Kabir, Z.D., Che, A., Fischer, D.K., Rice, R.C., Rizzo, B.K., Byrne, M., et al. (2017). Rescue of impaired sociability and anxiety-like behavior in adult cacna1c-deficient mice by pharmacologically targeting eIF2α. *Molecular psychiatry* 22(8), 1096-1109. doi: 10.1038/mp.2017.124.

Kacharmina, J.E., Job, C., Crino, P., and Eberwine, J. (2000). Stimulation of glutamate receptor protein synthesis and membrane insertion within isolated neuronal dendrites. *Proc Natl Acad Sci U S A* 97(21), 11545-11550. doi: 10.1073/pnas.97.21.11545.

Kacharmina, J.E., Job, C., Crino, P., and Eberwine, J. (2000). Stimulation of glutamate receptor protein synthesis and membrane insertion within isolated neuronal dendrites. *Proc Natl Acad Sci U S A* 97(21), 11545-11550. doi: 10.1073/pnas.97.21.11545.

Kaech, S., and Banker, G. (2006). Culturing hippocampal neurons. *Nat. Protocols* 1(5), 2406-2415.

Kafitz, K.W., Rose, C.R., Thoenen, H., and Konnerth, A. (1999). Neurotrophin-evoked rapid excitation through TrkB receptors. *Nature* 401(6756), 918-921. doi: 10.1038/44847.

Kajiwara, R., Tominaga, Y., and Tominaga, T. (2019). Network Plasticity Involved in the Spread of Neural Activity Within the Rhinal Cortices as Revealed by Voltage-Sensitive Dye Imaging in Mouse Brain Slices. *Frontiers in Cellular Neuroscience* 13(20). doi: 10.3389/fncel.2019.00020.

Kakegawa, W., Tsuzuki, K., Yoshida, Y., Kameyama, K., and Ozawa, S. (2004). Input- and subunit-specific AMPA receptor trafficking underlying long-term potentiation at hippocampal CA3 synapses. *European Journal of Neuroscience* 20(1), 101-110. doi: 10.1111/j.1460-9568.2004.03461.x.

Kamal, A., Stokin, G.B., Yang, Z., Xia, C.H., and Goldstein, L.S. (2000). Axonal transport of amyloid precursor protein is mediated by direct binding to the kinesin light chain subunit of kinesin-I. *Neuron* 28(2), 449-459. doi: 10.1016/s0896-6273(00)00124-0.

Kamenetz, F., Tomita, T., Hsieh, H., Seabrook, G., Borchelt, D., Iwatsubo, T., et al. (2003). APP Processing and Synaptic Function. *Neuron* 37(6), 925-937. doi: https://doi.org/10.1016/S0896-6273(03)00124-7.

Kamiya, H., and Zucker, R.S. (1994). Residual Ca^{2+} and short-term synaptic plasticity. *Nature* 371(6498), 603-606. doi: 10.1038/371603a0.

Kang, H., and Schuman, E.M. (1995). Long-lasting neurotrophin-induced enhancement of synaptic transmission in the adult hippocampus. *Science* 267(5204), 1658-1662.

Kato, K., Li, S.T., and Zorumski, C.F. (1999). Modulation of long-term potentiation induction in the hippocampus by N-methyl-D-aspartate-mediated presynaptic inhibition. *Neuroscience* 92(4), 1261-1272. doi: 10.1016/s0306-4522(99)00080-9.

Kato, N., and Yoshimura, H. (1993). Facilitatory effects of substance P on the susceptibility to long-term potentiation in the visual cortex of adult rats. *Brain Research* 617(2), 353-356. doi: https://doi.org/10.1016/0006-8993(93)91105-2.

Katsumaru, H., Kosaka, T., Heizmann, C.W., and Hama, K. (1988). Immunocytochemical study of GABAergic neurons containing the calcium-binding protein parvalbumin in the rat hippocampus. *Experimental Brain Research* 72(2), 347-362. doi: 10.1007/BF00250256.

Katz, B., and Miledi, R. (1968). The role of calcium in neuromuscular facilitation. *J Physiol* 195(2), 481-492.

Katz, D. (1920). *Psychologische versuche mit amputierten.* Barth.

Kauderer, B.S., and Kandel, E.R. (2000). Capture of a protein synthesis-dependent component of long-term depression. *Proceedings of the National Academy of Sciences of the United States of America* 97(24), 13342-13347. doi: 10.1073/pnas.97.24.13342.

Kawashima, T., Okuno, H., Nonaka, M., Adachi-Morishima, A., Kyo, N., Okamura, M., et al. (2009). Synaptic activity-responsive element in the Arc/Arg3.1 promoter essential for synapse-to-nucleus signaling in activated neurons. *Proceedings of the National Academy of Sciences of the United States of America* 106(1), 316-321. doi: 10.1073/pnas.0806518106.

Kazim, S.F., and Iqbal, K. (2016). Neurotrophic factor small-molecule mimetics mediated neuroregeneration and synaptic repair: emerging therapeutic modality for Alzheimer's disease. *Molecular Neurodegeneration* 11(1), 50. doi: 10.1186/s13024-016-0119-y.

Keifer, J., Tiwari, N.K., Buse, L., and Zheng, Z. (2017). Subunit-specific synaptic delivery of AMPA receptors by auxiliary chaperone proteins TARPγ8 and GSG1L in classical conditioning. *Neuroscience letters* 645, 53-59. doi: 10.1016/j.neulet.2017.02.041.

Keifer, J., Zheng, Z., and Mokin, M. (2008). Synaptic localization of GluR4-containing AMPARs and Arc during acquisition, extinction, and reacquisition of in vitro classical conditioning. *Neurobiology of Learning and Memory* 90(2), 301-308. doi: https://doi.org/10.1016/j.nlm.2008.04.011.

Keihan Falsafi, S., Ghafari, M., Pollak, A., Höger, H., and Lubec, G. (2012). Hippocampal AMPA-type receptor complexes containing GluR3 and GluR4 are paralleling training in the Multiple T-Maze. *Neurochemistry International* 60(4), 425-430. doi: https://doi.org/10.1016/j.neuint.2012.01.005.

Keller, D., Erö, C., and Markram, H. (2018). Cell Densities in the Mouse Brain: A Systematic Review. *Frontiers in neuroanatomy* 12, 83-83. doi: 10.3389/fnana.2018.00083.

Kelly, A., Conroy, S., and Lynch, M.A. (1998). Evidence that nerve growth factor plays a role in long-term potentiation in the rat dentate gyrus. *Neuropharmacology* 37(4-5), 561-570. doi: 10.1016/s0028-3908(98)00048-3.

Kelly, B.T., Graham, S.C., Liska, N., Dannhauser, P.N., Höning, S., Ungewickell, E.J., et al. (2014). Clathrin adaptors. AP2 controls clathrin polymerization with a membrane-activated switch. *Science (New York, N.Y.)* 345(6195), 459-463. doi: 10.1126/science.1254836.

Kemp, A., and Manahan-Vaughan, D. (2007). Hippocampal long-term depression: master or minion in declarative memory processes? *Trends Neurosci* 30(3), 111-118. doi: 10.1016/j.tins.2007.01.002.

Kemp, A., Tischmeyer, W., and Manahan-Vaughan, D. (2013). Learning-facilitated long-term depression requires activation of the immediate early gene, c-fos, and is transcription dependent. *Behavioural Brain Research* 254, 83-91. doi: https://doi.org/10.1016/j.bbr.2013.04.036.

Kennedy, F. (1898). On the experimental investigation of memory. *Psychological Review* 5(5), 477.

Kennedy, M.B., Bennett, M.K., and Erondu, N.E. (1983). Biochemical and immunochemical evidence that the "major postsynaptic density protein" is a subunit of a calmodulin-dependent protein kinase. *Proc Natl Acad Sci U S A* 80(23), 7357-7361. doi: 10.1073/pnas.80.23.7357.

Kennedy, M.J., and Ehlers, M.D. (2011). Mechanisms and function of dendritic exocytosis. *Neuron* 69(5), 856-875. doi: 10.1016/j.neuron.2011.02.032.

Kennedy, M.J., Davison, I.G., Robinson, C.G., and Ehlers, M.D. (2010). Syntaxin-4 Defines a Domain for Activity-Dependent Exocytosis in Dendritic Spines. *Cell* 141(3), 524-535. doi: 10.1016/j.cell.2010.02.042.

Keralapurath, M.M., Clark, J.K., Hammond, S., and Wagner, J.J. (2014). Cocaine- or stress-induced metaplasticity of LTP in the dorsal and ventral hippocampus. *Hippocampus* 24(5), 577-590. doi: 10.1002/hipo.22250.

Kerr, J.M., and Blanpied, T.A. (2012). Subsynaptic AMPA receptor distribution is acutely regulated by actin-driven reorganization of the postsynaptic density. *J Neurosci* 32(2), 658-673. doi: 10.1523/jneurosci.2927-11.2012.

Ketting, R.F. (2011). The many faces of RNAi. *Dev Cell* 20(2), 148-161. doi: 10.1016/j.devcel.2011.01.012.

Khan, G.M., Tong, M., Jhun, M., Arora, K., and Nichols, R.A. (2010). beta-Amyloid activates presynaptic alpha7 nicotinic acetylcholine receptors reconstituted into a model nerve cell system: involvement of lipid rafts. *Eur J Neurosci* 31(5), 788-796. doi: 10.1111/j.1460-9568.2010.07116.x.

Khan, M.I., Ostadhadi, S., Zolfaghari, S., Ejtemaei Mehr, S., Hassanzadeh, G., and Dehpour, A.R. (2016). The involvement of NMDA receptor/NO/cGMP pathway in the antidepressant like effects of baclofen in mouse force swimming test. *Neurosci Lett* 612, 52-61. doi: 10.1016/j.neulet.2015.12.006.

Kim, C.-H., Chung, H.J., Lee, H.-K., and Huganir, R.L. (2001a). Interaction of the AMPA receptor subunit GluR2/3 with PDZ domains regulates hippocampal long-term depression. *Proceedings of the National Academy of Sciences* 98(20), 11725-11730. doi: 10.1073/pnas.211132798.

Kim, C.H., and Lisman, J.E. (1999). A role of actin filament in synaptic transmission and long-term potentiation. *J Neurosci* 19(11), 4314-4324.

Kim, E., Naisbitt, S., Hsueh, Y.P., Rao, A., Rothschild, A., Craig, A.M., et al. (1997). GKAP, a novel synaptic protein that interacts with the guanylate kinase-like domain of the PSD-95/SAP90 family of channel clustering molecules. *J Cell Biol* 136(3), 669-678.

Kim, M., Suh, J., Romano, D., Truong, M.H., Mullin, K., Hooli, B., et al. (2009). Potential late-onset Alzheimer's disease-associated mutations in the ADAM10 gene attenuate {alpha}-secretase activity. *Hum Mol Genet* 18(20), 3987-3996. doi: 10.1093/hmg/ddp323.

Kim, S., and Ziff, E.B. (2014). Calcineurin Mediates Synaptic Scaling Via Synaptic Trafficking of Ca^{2+}-Permeable AMPA Receptors. *PLOS Biology* 12(7), e1001900. doi: 10.1371/journal.pbio.1001900.

Kim, V.N., Han, J., and Siomi, M.C. (2009). Biogenesis of small RNAs in animals. *Nature Reviews Molecular Cell Biology* 10(2), 126-139. doi: 10.1038/nrm2632.

Kirkwood, A., Rioult, M.G., and Bear, M.F. (1996). Experience-dependent modification of synaptic plasticity in visual cortex. *Nature* 381(6582), 526-528. doi: 10.1038/381526a0.

Kirschner, L.S., Yin, Z., Jones, G.N., and Mahoney, E. (2009). Mouse models of altered protein kinase A signaling. *Endocr Relat Cancer* 16(3), 773-793. doi: 10.1677/erc-09-0068.

Kirwan, C.B., Bayley, P.J., Galvan, V.V., and Squire, L.R. (2008). Detailed recollection of remote autobiographical memory after damage to the medial temporal lobe. *Proc Natl Acad Sci U S A* 105(7), 2676-2680. doi: 10.1073/pnas.0712155105.

Kiselycznyk, C., Zhang, X., Huganir, R.L., Holmes, A., and Svenningsson, P. (2013). Reduced phosphorylation of GluA1 subunits relates to anxiety-like behaviours in mice. *The international journal of neuropsychopharmacology* 16(4), 919-924. doi: 10.1017/S1461145712001174.

Kisselev, A.F., and Goldberg, A.L. (2005). "Monitoring Activity and Inhibition of 26S Proteasomes with Fluorogenic Peptide Substrates," in *Methods in Enzymology*. Academic Press), 364-378.

Kitajima, T., and Hara, K.-i. (1991). A model of the mechanism of cooperativity and associativity of long-term potentiation in the Hippocampus: a fundamental mechanism of associative memory and learning. *Biological Cybernetics* 64(5), 365-371. doi: 10.1007/BF00224703.

Klein, R.C., and Yakel, J.L. (2006). Functional somato-dendritic alpha7-containing nicotinic acetylcholine receptors in the rat basolateral amygdala complex. *J Physiol* 576(Pt 3), 865-872. doi: 10.1113/jphysiol.2006.118232.

Kleppisch, T., Wolfsgruber, W., Feil, S., Allmann, R., Wotjak, C.T., Goebbels, S., et al. (2003). Hippocampal cGMP-dependent protein kinase I supports an age- and protein synthesis-dependent component of long-term potentiation but is not essential for spatial reference and contextual memory. *J Neurosci* 23(14), 6005-6012.

Klevanski, M., Herrmann, U., Weyer, S.W., Fol, R., Cartier, N., Wolfer, D.P., et al. (2015). The APP Intracellular Domain Is Required for Normal Synaptic Morphology, Synaptic Plasticity, and Hippocampus-Dependent Behavior. *J Neurosci* 35(49), 16018-16033. doi: 10.1523/jneurosci.2009-15.2015.

Koffie, R.M., Meyer-Luehmann, M., Hashimoto, T., Adams, K.W., Mielke, M.L., Garcia-Alloza, M., et al. (2009). Oligomeric amyloid beta associates with postsynaptic densities and correlates with excitatory synapse loss near senile plaques. *Proc Natl Acad Sci U S A* 106(10), 4012-4017. doi: 10.1073/pnas.0811698106.

Koganezawa, N., Hanamura, K., Sekino, Y., and Shirao, T. (2017). The role of drebrin in dendritic spines. *Molecular and cellular neurosciences* 84, 85-92. doi: 10.1016/j.mcn.2017.01.004.

Koh, D.S., Geiger, J.R., Jonas, P., and Sakmann, B. (1995). Ca(2+)-permeable AMPA and NMDA receptor channels in basket cells of rat hippocampal dentate gyrus. *The Journal of Physiology* 485(2), 383-402. doi: 10.1113/jphysiol.1995.sp020737.

Köhr, G. (2006). NMDA receptor function: subunit composition versus spatial distribution. *Cell and Tissue Research* 326(2), 439-446. doi: 10.1007/s00441-006-0273-6.

Koike-Tani, M., Kanda, T., Saitoh, N., Yamashita, T., and Takahashi, T. (2008). Involvement of AMPA receptor desensitization in short-term synaptic depression at the calyx of Held in developing rats. *The Journal of Physiology* 586(Pt 9), 2263-2275. doi: 10.1113/jphysiol.2007.142547.

Koike, M.A., Lin, A.J., Pham, J., Nguyen, E., Yeh, J.J., Rahimian, R., et al. (2012). APP knockout mice experience acute mortality as the result of ischemia. *PLoS One* 7(8), e42665. doi: 10.1371/journal.pone.0042665.

Kojima, N., and Shirao, T. (2007). Synaptic dysfunction and disruption of postsynaptic drebrin–actin complex: A study of neurological disorders accompanied by cognitive deficits. *Neuroscience Research* 58(1), 1-5. doi: https://doi.org/10.1016/j.neures.2007.02.003.

Konagaya, S., Ando, T., Yamauchi, T., Suemori, H., and Iwata, H. (2015). Long-term maintenance of human induced pluripotent stem cells by automated cell culture system. *Scientific Reports* 5(1), 16647. doi: 10.1038/srep16647.

Konen, L.M., Wright, A.L., Royle, G.A., Morris, G.P., Lau, B.K., Seow, P.W., et al. (2020). A new mouse line with reduced GluA2 Q/R site RNA editing exhibits loss of dendritic spines, hippocampal CA1-neuron loss, learning and memory impairments and NMDA receptor-independent seizure vulnerability. *Molecular Brain* 13(1), 27. doi: 10.1186/s13041-020-0545-1.

Koo, E.H., Sisodia, S.S., Archer, D.R., Martin, L.J., Weidemann, A., Beyreuther, K., et al. (1990). Precursor of amyloid protein in Alzheimer disease undergoes fast anterograde axonal transport. *Proc Natl Acad Sci U S A* 87(4), 1561-1565. doi: 10.1073/pnas.87.4.1561.

Kopec, A.M., Philips, G.T., and Carew, T.J. (2015). Distinct Growth Factor Families Are Recruited in Unique Spatiotemporal Domains during Long-Term Memory Formation in Aplysia californica. *Neuron* 86(5), 1228-1239. doi: 10.1016/j.neuron.2015.04.025.

Kopec, C.D., Li, B., Wei, W., Boehm, J., and Malinow, R. (2006). Glutamate Receptor Exocytosis and Spine Enlargement during Chemically Induced Long-Term Potentiation. *The Journal of Neuroscience* 26(7), 2000-2009. doi: 10.1523/jneurosci.3918-05.2006.

Kopec, C.D., Real, E., Kessels, H.W., and Malinow, R. (2007). GluR1 links structural and functional plasticity at excitatory synapses. *J Neurosci* 27(50), 13706-13718. doi: 10.1523/jneurosci.3503-07.2007.

Korb, E., and Finkbeiner, S. (2011). Arc in synaptic plasticity: from gene to behavior. *Trends in neurosciences* 34(11), 591-598. doi: 10.1016/j.tins.2011.08.007.

Korb, E., Wilkinson, C.L., Delgado, R.N., Lovero, K.L., and Finkbeiner, S. (2013). Arc in the nucleus regulates PML-dependent GluA1 transcription and homeostatic plasticity. *Nat Neurosci* 16(7), 874-883. doi: 10.1038/nn.3429.

Kornau, H., Schenker, L., Kennedy, M., and Seeburg, P. (1995). Domain interaction between NMDA receptor subunits and the postsynaptic density protein PSD-95. *Science* 269(5231), 1737-1740. doi: 10.1126/science.7569905.

Korobova, F., and Svitkina, T. (2010). Molecular architecture of synaptic actin cytoskeleton in hippocampal neurons reveals a mechanism of dendritic spine morphogenesis. *Mol Biol Cell* 21(1), 165-176. doi: 10.1091/mbc.E09-07-0596.

Korte, M., Carroll, P., Wolf, E., Brem, G., Thoenen, H., and Bonhoeffer, T. (1995). Hippocampal long-term potentiation is impaired in mice lacking brain-derived neurotrophic factor. *Proc Natl Acad Sci U S A* 92(19), 8856-8860. doi: 10.1073/pnas.92.19.8856.

Korte, M., Griesbeck, O., Gravel, C., Carroll, P., Staiger, V., Thoenen, H., et al. (1996). Virus-mediated gene transfer into hippocampal CA1 region restores long-term potentiation in brain-derived neurotrophic factor mutant mice. *Proceedings of the National Academy of Sciences of the United States of America* 93(22), 12547-12552. doi: 10.1073/pnas.93.22.12547.

Kosaka T, Katsumaru H, Hama K, Wu JY, Heizmann CW (1987) GABAergic neurons containing the Ca2+-binding protein parvalbumin in the rat hippocampus and dentate gyrus. Brain research 419:119-130.

Koszegi, Z., Fiuza, M., and Hanley, J.G. (2017). Endocytosis and lysosomal degradation of GluA2/3 AMPARs in response to oxygen/glucose deprivation in hippocampal but not cortical neurons. *Sci Rep* 7(1), 12318. doi: 10.1038/s41598-017-12534-w.

Krijnse-Locker, J., Parton, R.G., Fuller, S.D., Griffiths, G., and Dotti, C.G. (1995). The organization of the endoplasmic reticulum and the intermediate compartment in cultured rat hippocampal neurons. *Mol Biol Cell* 6(10), 1315-1332. doi: 10.1091/mbc.6.10.1315.

Kristensen, S.E., Thomsen, M.S., Hansen, H.H., Timmermann, D.B., Hay-Schmidt, A., and Mikkelsen, J.D. (2007). The α7 nicotinic receptor agonist SSR180711 increases activity regulated cytoskeleton protein (Arc) gene expression in the prefrontal cortex of the rat. *Neuroscience letters* 418(2), 154-158.

Kroker, K.S., Moreth, J., Kussmaul, L., Rast, G., and Rosenbrock, H. (2013). Restoring long-term potentiation impaired by amyloid-beta oligomers: Comparison of an acetylcholinesterase inhibitior and selective neuronal nicotinic receptor agonists. *Brain Research Bulletin* 96, 28-38. doi: https://doi.org/10.1016/j.brainresbull.2013.04.006.

Krucker, T., Siggins, G.R., and Halpain, S. (2000). Dynamic actin filaments are required for stable long-term potentiation (LTP) in area CA1 of the hippocampus. *Proc Natl Acad Sci U S A* 97(12), 6856-6861. doi: 10.1073/pnas.100139797.

Krug, M., Lossner, B., and Ott, T. (1984). Anisomycin blocks the late phase of long-term potentiation in the dentate gyrus of freely moving rats. *Brain Res Bull* 13(1), 39-42.

Kuba, K., and Kumamoto, E. (1986). Long-term potentiation of transmitter release induced by adrenaline in bull-frog sympathetic ganglia. *J Physiol* 374, 515-530. doi: 10.1113/jphysiol.1986.sp016095.

Kuipers, S.D., Trentani, A., Tiron, A., Mao, X., Kuhl, D., and Bramham, C.R. (2016). BDNF-induced LTP is associated with rapid Arc/Arg3.1-dependent enhancement in adult hippocampal neurogenesis. *Scientific Reports* 6(1), 21222. doi: 10.1038/srep21222.

Kullmann, D.M., and Nicoll, R.A. (1992). Long-term potentiation is associated with increases in quantal content and quantal amplitude. *Nature* 357(6375), 240-244. doi: 10.1038/357240a0.

Kumamoto, E., and Kuba, K. (1987). Mechanisms regulating the adrenaline-induced long-term potentiation in bullfrog sympathetic ganglia. *Pflugers Arch* 408(6), 573-577. doi: 10.1007/bf00581158.

Kumar, V., Fahey, P.G., Jong, Y.-J.I., Ramanan, N., and O'Malley, K.L. (2012). Activation of intracellular metabotropic glutamate receptor 5 in striatal neurons leads to up-regulation of genes associated with sustained synaptic transmission including Arc/Arg3. 1 protein. *Journal of Biological Chemistry* 287(8), 5412-5425.

Kundu, A., Milosch, N., Antonietti, P., Baumkotter, F., Zymny, A., Muller, U.C., et al. (2016). Modulation of BAG3 Expression and Proteasomal Activity by sAPPalpha Does Not Require Membrane-Tethered Holo-APP. *Mol Neurobiol* 53(9), 5985-5994. doi: 10.1007/s12035-015-9501-y.

Kurup, P., Zhang, Y., Xu, J., Venkitaramani, D.V., Haroutunian, V., Greengard, P., et al. (2010). Abeta-mediated NMDA receptor endocytosis in Alzheimer's disease involves ubiquitination of the tyrosine phosphatase STEP61. *J Neurosci* 30(17), 5948-5957. doi: 10.1523/jneurosci.0157-10.2010.

Kusuda, R., Carreira, E.U., Ulloa, L., Cunha, F.Q., Kanashiro, A., and Cunha, T.M. (2020). Choline attenuates inflammatory hyperalgesia activating nitric oxide/cGMP/ATP-sensitive potassium channels pathway. *Brain research* 1727, 146567-146567. doi: 10.1016/j.brainres.2019.146567.

Kusumi, A., Sako, Y., and Yamamoto, M. (1993). Confined lateral diffusion of membrane receptors as studied by single particle tracking (nanovid microscopy). Effects of calcium-induced differentiation in cultured epithelial cells. *Biophys J* 65(5), 2021-2040. doi: 10.1016/s0006-3495(93)81253-0.

Kwon, H.B., and Sabatini, B.L. (2011). Glutamate induces de novo growth of functional spines in developing cortex. *Nature* 474(7349), 100-104. doi: 10.1038/nature09986.

Kwon, O.B., Paredes, D., Gonzalez, C.M., Neddens, J., Hernandez, L., Vullhorst, D., et al. (2008). Neuregulin-1 regulates LTP at CA1 hippocampal synapses through activation of dopamine D4 receptors. *Proc Natl Acad Sci U S A* 105(40), 15587-15592. doi: 10.1073/pnas.0805722105.

Kwon, Sung E., and Chapman, Edwin R. (2011). Synaptophysin Regulates the Kinetics of Synaptic Vesicle Endocytosis in Central Neurons. *Neuron* 70(5), 847-854. doi: 10.1016/j.neuron.2011.04.001.

Kyrke-Smith, M., Volk, L.J., Cooke, S.F., Bear, M.F., Huganir, R.L., and Shepherd, J.D. (2020). The immediate early gene Arc is not required for hippocampal long-term potentiation. *bioRxiv*, 2020.2001.2003.893305. doi: 10.1101/2020.01.03.893305.

Lacor, P.N., Buniel, M.C., Chang, L., Fernandez, S.J., Gong, Y., Viola, K.L., et al. (2004). Synaptic targeting by Alzheimer's-related amyloid beta oligomers. *J Neurosci* 24(45), 10191-10200. doi: 10.1523/jneurosci.3432-04.2004.

Lahiri, D.K., Chen, D., Maloney, B., Holloway, H.W., Yu, Q.S., Utsuki, T., et al. (2007). The experimental Alzheimer's disease drug posiphen [(+)-phenserine] lowers amyloid-beta peptide levels in cell culture and mice. *J Pharmacol Exp Ther* 320(1), 386-396. doi: 10.1124/jpet.106.112102.

Lahiri, D.K., Maloney, B., Long, J.M., and Greig, N.H. (2014). Lessons from a BACE1 inhibitor trial: off-site but not off base. *Alzheimers Dement* 10(5 Suppl), S411-419. doi: 10.1016/j.jalz.2013.11.004.

Lai, R., Albala, B., Kaplow, J.M., Aluri, J., Yen, M., and Satlin, A. (2012). O1-06-05: First-in-human study of E2609, a novel BACE1 inhibitor, demonstrates prolonged reductions in plasma beta-amyloid levels after single dosing. *Alzheimer's & Dementia* 8(4S_Part_3), P96-P96. doi: 10.1016/j.jalz.2012.05.237.

Lammich, S., Kojro, E., Postina, R., Gilbert, S., Pfeiffer, R., Jasionowski, M., et al. (1999). Constitutive and regulated α-secretase cleavage of Alzheimer's amyloid precursor protein by a disintegrin metalloprotease. *Proc Natl Acad Sci U S A* 96(7), 3922-3927.

Lanahan, A., and Worley, P. (1998). Immediate-early genes and synaptic function. *Neurobiol Learn Mem* 70(1-2), 37-43. doi: 10.1006/nlme.1998.3836.

Landen, J.W., Zhao, Q., Cohen, S., Borrie, M., Woodward, M., Billing, C.B., Jr., et al. (2013). Safety and pharmacology of a single intravenous dose of ponezumab in subjects with mild-to-moderate Alzheimer disease: a phase I, randomized, placebo-controlled, double-blind, dose-escalation study. *Clin Neuropharmacol* 36(1), 14-23. doi: 10.1097/WNF.0b013e31827db49b.

Langstrom, N.S., Anderson, J.P., Lindroos, H.G., Winbland, B., and Wallace, W.C. (1989). Alzheimer's disease-associated reduction of polysomal mRNA translation. *Molecular Brain Research* 5(4), 259-269. doi: https://doi.org/10.1016/0169-328X(89)90060-0.

Lannfelt, L., Basun, H., Wahlund, L.O., Rowe, B.A., and Wagner, S.L. (1995). Decreased alpha-secretase-cleaved amyloid precursor protein as a diagnostic marker for Alzheimer's disease. *Nat Med* 1(8), 829-832.

Lanté, F., Toledo-Salas, J.-C., Ondrejcak, T., Rowan, M.J., and Ulrich, D. (2011). Removal of synaptic Ca^{2+}-permeable AMPA receptors during sleep. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 31(11), 3953-3961. doi: 10.1523/JNEUROSCI.3210-10.2011.

Lasala, M., Fabiani, C., Corradi, J., Antollini, S., and Bouzat, C. (2019). Molecular Modulation of Human alpha7 Nicotinic Receptor by Amyloid-beta Peptides. *Front Cell Neurosci* 13, 37. doi: 10.3389/fncel.2019.00037.

Lauer, L., Vogt, A., Yeung, C.K., Knoll, W., and Offenhausser, A. (2002). Electrophysiological recordings of patterned rat brain stem slice neurons. *Biomaterials* 23(15), 3123-3130. doi: 10.1016/s0142-9612(02)00056-x.

Lavado, A., Lagutin, O.V., Chow, L.M., Baker, S.J., and Oliver, G. (2010). Prox1 is required for granule cell maturation and intermediate progenitor maintenance during brain neurogenesis. *PLoS Biol* 8(8). doi: 10.1371/journal.pbio.1000460.

Lazarevic, V., Fieńko, S., Andres-Alonso, M., Anni, D., Ivanova, D., Montenegro-Venegas, C., et al. (2017). Physiological Concentrations of Amyloid Beta Regulate Recycling of Synaptic Vesicles via Alpha7 Acetylcholine Receptor and CDK5/Calcineurin Signaling. *Frontiers in molecular neuroscience* 10, 221-221. doi: 10.3389/fnmol.2017.00221.

Leal, G., Comprido, D., and Duarte, C.B. (2014). BDNF-induced local protein synthesis and synaptic plasticity. *Neuropharmacology* 76 Pt C, 639-656. doi: 10.1016/j.neuropharm.2013.04.005.

Leal, K., and Klein, M. (2009). Direct enhancement of presynaptic calcium influx in presynaptic facilitation at Aplysia sensorimotor synapses. *Mol Cell Neurosci* 41(2), 247-257. doi: 10.1016/j.mcn.2009.03.004.

LeBlanc, A.C., Xue, R., and Gambetti, P. (1996). Amyloid precursor protein metabolism in primary cell cultures of neurons, astrocytes, and microglia. *J Neurochem* 66(6), 2300-2310. doi: 10.1046/j.1471-4159.1996.66062300.x.

LeBrasseur, N. (2005). The early neurite gets the axon. *The Journal of Cell Biology* 170(5), 698-698. doi: 10.1083/jcb1705rr2.

Lee, B.R., Ma, Y.-Y., Huang, Y.H., Wang, X., Otaka, M., Ishikawa, M., et al. (2013). Maturation of silent synapses in amygdala-accumbens projection contributes to incubation of cocaine craving. *Nature Neuroscience* 16(11), 1644-1651. doi: 10.1038/nn.3533.

Lee, H.-K., Barbarosie, M., Kameyama, K., Bear, M.F., and Huganir, R.L. (2000a). Regulation of distinct AMPA receptor phosphorylation sites during bidirectional synaptic plasticity. *Nature* 405(6789), 955-959. doi: 10.1038/35016089.

Lee, H.-K., Kameyama, K., Huganir, R.L., and Bear, M.F. (1998). NMDA Induces Long-Term Synaptic Depression and Dephosphorylation of the GluR1 Subunit of AMPA Receptors in Hippocampus. *Neuron* 21(5), 1151-1162. doi: https://doi.org/10.1016/S0896-6273(00)80632-7.

Lee, H.-K., Takamiya, K., Han, J.-S., Man, H., Kim, C.-H., Rumbaugh, G., et al. (2003). Phosphorylation of the AMPA Receptor GluR1 Subunit Is Required for Synaptic Plasticity and Retention of Spatial Memory. *Cell* 112(5), 631-643. doi: https://doi.org/10.1016/S0092-8674(03)00122-3.

Lee, H.K. (2012). Ca(2+)-permeable AMPA receptors in homeostatic synaptic plasticity. *Front Mol Neurosci* 5. doi: 10.3389/fnmol.2012.00017.

Lee, H.K., Takamiya, K., He, K., Song, L., and Huganir, R.L. (2010a). Specific roles of AMPA receptor subunit GluR1 (GluA1) phosphorylation sites in regulating synaptic plasticity in the CA1 region of hippocampus. *J Neurophysiol* 103(1), 479-489. doi: 10.1152/jn.00835.2009.

Lee, H.K., Takamiya, K., Kameyama, K., He, K., Yu, S., Rossetti, L., et al. (2007). Identification and characterization of a novel phosphorylation site on the GluR1 subunit of AMPA receptors. *Mol Cell Neurosci* 36(1), 86-94. doi: 10.1016/j.mcn.2007.06.003.

Lee, K., Park, T.I.-H., Heppner, P., Schweder, P., Mee, E.W., Dragunow, M., et al. (2020). Human in vitro systems for examining synaptic function and plasticity in the brain. *Journal of Neurophysiology* 123(3), 945-965. doi: 10.1152/jn.00411.2019.

Lee, K.J., Moussa, C.E., Lee, Y., Sung, Y., Howell, B.W., Turner, R.S., et al. (2010b). Beta amyloid-independent role of amyloid precursor protein in generation and maintenance of dendritic spines. *Neuroscience* 169(1), 344-356. doi: 10.1016/j.neuroscience.2010.04.078.

Lee, K.J., Park, I.S., Kim, H., Greenough, W.T., Pak, D.T.S., and Rhyu, I.J. (2013). Motor Skill Training Induces Coordinated Strengthening and Weakening between Neighboring Synapses. *The Journal of Neuroscience* 33(23), 9794-9799. doi: 10.1523/jneurosci.0848-12.2013.

Lee, K.S., Schottler, F., Oliver, M., and Lynch, G. (1980). Brief bursts of high-frequency stimulation produce two types of structural change in rat hippocampus. *Journal of Neurophysiology* 44(2), 247-258. doi: 10.1152/jn.1980.44.2.247.

Lee, L., Kosuri, P., and Arancio, O. (2014). Picomolar amyloid-beta peptides enhance spontaneous astrocyte calcium transients. *J Alzheimers Dis* 38(1), 49-62. doi: 10.3233/jad-130740.

Lee, S.H., Liu, L., Wang, Y.T., and Sheng, M. (2002). Clathrin Adaptor AP2 and NSF Interact with Overlapping Sites of GluR2 and Play Distinct Roles in AMPA Receptor Trafficking and Hippocampal LTD. *Neuron* 36(4), 661-674. doi: https://doi.org/10.1016/S0896-6273(02)01024-3.

Lee, S.J., Escobedo-Lozoya, Y., Szatmari, E.M., and Yasuda, R. (2009). Activation of CaMKII in single dendritic spines during long-term potentiation. *Nature* 458(7236), 299-304. doi: 10.1038/nature07842.

Lein, P.J., Barnhart, C.D., and Pessah, I.N. (2011). Acute hippocampal slice preparation and hippocampal slice cultures. *Methods in molecular biology (Clifton, N.J.)* 758, 115-134. doi: 10.1007/978-1-61779-170-3_8.

Lendvai, B., Stern, E.A., Chen, B., and Svoboda, K. (2000). Experience-dependent plasticity of dendritic spines in the developing rat barrel cortex in vivo. *Nature* 404(6780), 876-881. doi: 10.1038/35009107.

Leonard, A.S., Davare, M.A., Horne, M.C., Garner, C.C., and Hell, J.W. (1998). SAP97 is associated with the alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor GluR1 subunit. *J Biol Chem* 273(31), 19518-19524. doi: 10.1074/jbc.273.31.19518.

Leonard, A.S., Lim, I.A., Hemsworth, D.E., Horne, M.C., and Hell, J.W. (1999). Calcium/calmodulin-dependent protein kinase II is associated with the N-methyl-d-aspartate receptor. *Proceedings of the National Academy of Sciences* 96(6), 3239-3244. doi: 10.1073/pnas.96.6.3239.

Leonoudakis, D., Zhao, P., and Beattie, E.C. (2008). Rapid tumor necrosis factor alpha-induced exocytosis of glutamate receptor 2-lacking AMPA receptors to extrasynaptic plasma membrane potentiates excitotoxicity. *J Neurosci* 28(9), 2119-2130. doi: 10.1523/jneurosci.5159-07.2008.

Levy, R.B., and Aoki, C. (2002). Alpha7 nicotinic acetylcholine receptors occur at postsynaptic densities of AMPA receptor-positive and -negative excitatory synapses in rat sensory cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 22(12), 5001-5015. doi: 10.1523/JNEUROSCI.22-12-05001.2002.

Levy, W.B., and Steward, O. (1983). Temporal contiguity requirements for long-term associative potentiation/depression in the hippocampus. *Neuroscience* 8(4), 791-797. doi: https://doi.org/10.1016/0306-4522(83)90010-6.

Lewczuk, P., Kamrowski-Kruck, H., Peters, O., Heuser, I., Jessen, F., Popp, J., et al. (2010). Soluble amyloid precursor proteins in the cerebrospinal fluid as novel potential biomarkers of Alzheimer's disease: a multicenter study. *Mol Psychiatry* 15(2), 138-145. doi: 10.1038/mp.2008.84.

Li, H., Wang, B., Wang, Z., Guo, Q., Tabuchi, K., Hammer, R.E., et al. (2010). Soluble amyloid precursor protein (APP) regulates transthyretin and Klotho gene expression without rescuing the essential function of APP. *Proc Natl Acad Sci U S A* 107(40), 17362-17367. doi: 10.1073/pnas.1012568107.

Li, L.P., Dustrude, E.T., Haulcomb, M.M., Abreu, A.R., Fitz, S.D., Johnson, P.L., et al. (2018). PSD95 and nNOS interaction as a novel molecular target to modulate conditioned fear: relevance to PTSD. *Translational Psychiatry* 8(1), 155. doi: 10.1038/s41398-018-0208-5.

Li, N., Li, Y., Li, L.-J., Zhu, K., Zheng, Y., and Wang, X.-M. (2019). Glutamate receptor delocalization in postsynaptic membrane and reduced hippocampal synaptic plasticity in the early stage of Alzheimer’s disease. *Neural Regeneration Research* 14(6), 1037-1045. doi: 10.4103/1673-5374.250625.

Li, R., Lindholm, K., Yang, L.B., Yue, X., Citron, M., Yan, R., et al. (2004). Amyloid beta peptide load is correlated with increased beta-secretase activity in sporadic Alzheimer's disease patients. *Proc Natl Acad Sci U S A* 101(10), 3632-3637. doi: 10.1073/pnas.0205689101.

Li, S., Cullen, W.K., Anwyl, R., and Rowan, M.J. (2003). Dopamine-dependent facilitation of LTP induction in hippocampal CA1 by exposure to spatial novelty. *Nat Neurosci* 6(5), 526-531. doi: 10.1038/nn1049.

Li, S., Hong, S., Shepardson, N.E., Walsh, D.M., Shankar, G.M., and Selkoe, D. (2009). Soluble oligomers of amyloid Beta protein facilitate hippocampal long-term depression by disrupting neuronal glutamate uptake. *Neuron* 62(6), 788-801. doi: 10.1016/j.neuron.2009.05.012.

Li, S., Li, Z., Pei, L., Le, A.D., and Liu, F. (2012). The α7nACh-NMDA receptor complex is involved in cue-induced reinstatement of nicotine seeking. *J Exp Med* 209(12), 2141-2147. doi: 10.1084/jem.20121270.

Li, S., Nai, Q., Lipina, T.V., Roder, J.C., and Liu, F. (2013). α7nAchR/NMDAR coupling affects NMDAR function and object recognition. *Molecular brain* 6, 58-58. doi: 10.1186/1756-6606-6-58.

Li, X., and Wolf, M.E. (2011). Brain-derived neurotrophic factor rapidly increases AMPA receptor surface expression in rat nucleus accumbens. *The European journal of neuroscience* 34(2), 190-198. doi: 10.1111/j.1460-9568.2011.07754.x.

Li, X., and Wolf, M.E. (2011). Brain-derived neurotrophic factor rapidly increases AMPA receptor surface expression in rat nucleus accumbens. *The European journal of neuroscience* 34(2), 190-198. doi: 10.1111/j.1460-9568.2011.07754.x.

Li, Y.M., Lai, M.T., Xu, M., Huang, Q., DiMuzio-Mower, J., Sardana, M.K., et al. (2000). Presenilin 1 is linked with gamma-secretase activity in the detergent solubilized state. *Proceedings of the National Academy of Sciences of the United States of America* 97(11), 6138-6143. doi: 10.1073/pnas.110126897.

Lian, D., He, D., Wu, J., Liu, Y., Zhu, M., Sun, J., et al. (2016). Exogenous BDNF increases neurogenesis in the hippocampus in experimental Streptococcus pneumoniae meningitis. *Journal of Neuroimmunology* 294, 46-55. doi: 10.1016/j.jneuroim.2016.03.014.

Liao, D., Hessler, N.A., and Malinow, R. (1995). Activation of postsynaptically silent synapses during pairing-induced LTP in CA1 region of hippocampal slice. *Nature* 375(6530), 400-404. doi: 10.1038/375400a0.

Liao, D., Scannevin, R.H., and Huganir, R. (2001). Activation of silent synapses by rapid activity-dependent synaptic recruitment of AMPA receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 21(16), 6008-6017. doi: 10.1523/JNEUROSCI.21-16-06008.2001.

Liao, D., Zhang, X., O'Brien, R., Ehlers, M.D., and Huganir, R.L. (1999). Regulation of morphological postsynaptic silent synapses in developing hippocampal neurons. *Nat Neurosci* 2(1), 37-43. doi: 10.1038/4540.

Liao, M.-C., Muratore, C.R., Gierahn, T.M., Sullivan, S.E., Srikanth, P., De Jager, P.L., et al. (2016). Single-Cell Detection of Secreted Aβ and sAPPα from Human IPSC-Derived Neurons and Astrocytes.

The Journal of neuroscience : the official journal of the Society for Neuroscience 36(5), 1730-1746. doi: 10.1523/JNEUROSCI.2735-15.2016.

Lin, B., Kramár, E.A., Bi, X., Brucher, F.A., Gall, C.M., and Lynch, G. (2005). Theta stimulation polymerizes actin in dendritic spines of hippocampus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 25(8), 2062-2069. doi: 10.1523/JNEUROSCI.4283-04.2005.

Lin, D.-T., and Huganir, R.L. (2007). PICK1 and Phosphorylation of the Glutamate Receptor 2 (GluR2) AMPA Receptor Subunit Regulates GluR2 Recycling after NMDA Receptor-Induced Internalization. *The Journal of Neuroscience* 27(50), 13903-13908. doi: 10.1523/jneurosci.1750-07.2007.

Lin, D.T., Makino, Y., Sharma, K., Hayashi, T., Neve, R., Takamiya, K., et al. (2009). Regulation of AMPA receptor extrasynaptic insertion by 4.1N, phosphorylation and palmitoylation. *Nat Neurosci* 12(7), 879-887. doi: 10.1038/nn.2351.

Lin, Y.-C., and Redmond, L. (2008). CaMKIIβ binding to stable F-actin in vivo regulates F-actin filament stability. *Proceedings of the National Academy of Sciences* 105(41), 15791-15796. doi: 10.1073/pnas.0804399105.

Lindecke, A., Korte, M., Zagrebelsky, M., Horejschi, V., Elvers, M., Widera, D., et al. (2006). Long-term depression activates transcription of immediate early transcription factor genes: involvement of serum response factor/Elk-1. *European Journal of Neuroscience* 24(2), 555-563. doi: 10.1111/j.1460-9568.2006.04909.x.

Ling, D.S.F., Benardo, L.S., Serrano, P.A., Blace, N., Kelly, M.T., Crary, J.F., et al. (2002). Protein kinase Mζ is necessary and sufficient for LTP maintenance. *Nature Neuroscience* 5, 295. doi: 10.1038/nn829

Link, W., Konietzko, U., Kauselmann, G., Krug, M., Schwanke, B., Frey, U., et al. (1995). Somatodendritic expression of an immediate early gene is regulated by synaptic activity. *Proc Natl Acad Sci U S A* 92(12), 5734-5738.

Lisman, J. (1989). A mechanism for the Hebb and the anti-Hebb processes underlying learning and memory. *Proceedings of the National Academy of Sciences of the United States of America* 86(23), 9574-9578. doi: 10.1073/pnas.86.23.9574.

Lisman, J. (1994). The CaM kinase II hypothesis for the storage of synaptic memory. *Trends Neurosci* 17(10), 406-412.

Lisman, J., Buzsáki, G., Eichenbaum, H., Nadel, L., Ranganath, C., and Redish, A.D. (2017). Viewpoints: how the hippocampus contributes to memory, navigation and cognition. *Nature Neuroscience* 20(11), 1434-1447. doi: 10.1038/nn.4661.

Lisman, J., Schulman, H., and Cline, H. (2002). The molecular basis of CaMKII function in synaptic and behavioural memory. *Nat Rev Neurosci* 3(3), 175-190. doi: 10.1038/nrn753.

Lisman, J., Yasuda, R., and Raghavachari, S. (2012). Mechanisms of CaMKII action in long-term potentiation. *Nat Rev Neurosci* 13(3), 169-182. doi: 10.1038/nrn3192.

Lisman, J.E. (1985). A mechanism for memory storage insensitive to molecular turnover: a bistable autophosphorylating kinase. *Proc Natl Acad Sci U S A* 82(9), 3055-3057. doi: 10.1073/pnas.82.9.3055.

Lissin, D.V., Gomperts, S.N., Carroll, R.C., Christine, C.W., Kalman, D., Kitamura, M., et al. (1998). Activity differentially regulates the surface expression of synaptic AMPA and NMDA glutamate receptors. *Proc Natl Acad Sci U S A* 95(12), 7097-7102.

Lista, S., Dubois, B., and Hampel, H. (2015). Paths to Alzheimer's disease prevention: from modifiable risk factors to biomarker enrichment strategies. *J Nutr Health Aging* 19(2), 154-163. doi: 10.1007/s12603-014-0515-3.

Liu, P.Z., and Nusslock, R. (2018). Exercise-Mediated Neurogenesis in the Hippocampus via BDNF. *Front Neurosci* 12, 52. doi: 10.3389/fnins.2018.00052.

Liu, Q., Kawai, H., and Berg, D.K. (2001). beta -Amyloid peptide blocks the response of alpha 7-containing nicotinic receptors on hippocampal neurons. *Proc Natl Acad Sci U S A* 98(8), 4734-4739. doi: 10.1073/pnas.081553598.

Liu, R.-Y., Cleary, L.J., and Byrne, J.H. (2011). The Requirement for Enhanced CREB1 Expression in Consolidation of Long-Term Synaptic Facilitation and Long-Term Excitability in Sensory Neurons of Aplysia. *The Journal of Neuroscience* 31(18), 6871-6879. doi: 10.1523/jneurosci.5071-10.2011.

Liu, S.-B., Zhang, M.-M., Cheng, L.-F., Shi, J., Lu, J.-S., and Zhuo, M. (2015a). Long-term upregulation of cortical glutamatergic AMPA receptors in a mouse model of chronic visceral pain. *Molecular Brain* 8(1), 76. doi: 10.1186/s13041-015-0169-z.

Liu, S.-Q.J., and Cull-Candy, S.G. (2000). Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. *Nature* 405, 454. doi: 10.1038/35013064.

Liu, S., Rao, Y., and Daw, N. (2003). Roles of Protein Kinase A and Protein Kinase G in Synaptic Plasticity in the Visual Cortex. *Cerebral Cortex* 13(8), 864-869. doi: 10.1093/cercor/13.8.864.

Liu, S.J., and Cull-Candy, S.G. (2005). Subunit interaction with PICK and GRIP controls Ca^{2+} permeability of AMPARs at cerebellar synapses. *Nature Neuroscience* 8(6), 768-775. doi: 10.1038/nn1468.

Liu, S.J., Gasperini, R., Foa, L., and Small, D.H. (2010). Amyloid-beta decreases cell-surface AMPA receptors by increasing intracellular calcium and phosphorylation of GluR2. *J Alzheimers Dis* 21(2), 655-666. doi: 10.3233/jad-2010-091654.

Liu, X.-L., Luo, L., Mu, R.-H., Liu, B.-B., Geng, D., Liu, Q., et al. (2015b). Fluoxetine regulates mTOR signalling in a region-dependent manner in depression-like mice. *Scientific Reports* 5(1), 16024. doi: 10.1038/srep16024.

Liu, Y., Zhou, L.-J., Wang, J., Li, D., Ren, W.-J., Peng, J., et al. (2017). TNF-α Differentially Regulates Synaptic Plasticity in the Hippocampus and Spinal Cord by Microglia-Dependent Mechanisms after Peripheral Nerve Injury. *The Journal of Neuroscience* 37(4), 871-881. doi: 10.1523/jneurosci.2235-16.2016.

Liu, Y., Zhou, Q.-X., Hou, Y.-Y., Lu, B., Yu, C., Chen, J., et al. (2012). Actin polymerization-dependent increase in synaptic Arc/Arg3.1 expression in the amygdala is crucial for the expression of aversive memory associated with drug withdrawal. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 32(35), 12005-12017. doi: 10.1523/JNEUROSCI.0871-12.2012.

Livingstone, R.W., Elder, M.K., Barrett, M.C., Westlake, C.M., Peppercorn, K., Tate, W.P., et al. (2019). Secreted Amyloid Precursor Protein-Alpha Promotes Arc Protein Synthesis in Hippocampal Neurons. *Frontiers in Molecular Neuroscience* 12(198). doi: 10.3389/fnmol.2019.00198.

Lledo, P.M., Hjelmstad, G.O., Mukherji, S., Soderling, T.R., Malenka, R.C., and Nicoll, R.A. (1995). Calcium/calmodulin-dependent kinase II and long-term potentiation enhance synaptic transmission by the same mechanism. *Proceedings of the National Academy of Sciences* 92(24), 11175-11179. doi: 10.1073/pnas.92.24.11175.

Loane, D.J., Pocivavsek, A., Moussa, C.E., Thompson, R., Matsuoka, Y., Faden, A.I., et al. (2009). Amyloid precursor protein secretases as therapeutic targets for traumatic brain injury. *Nat Med* 15(4), 377-379. doi: 10.1038/nm.1940.

Lock, J.G., and Stow, J.L. (2005). Rab11 in recycling endosomes regulates the sorting and basolateral transport of E-cadherin. *Mol Biol Cell* 16(4), 1744-1755. doi: 10.1091/mbc.e04-10-0867.

Loebrich, S., Djukic, B., Tong, Z.J., Cottrell, J.R., Turrigiano, G.G., and Nedivi, E. (2013). Regulation of glutamate receptor internalization by the spine cytoskeleton is mediated by its PKA-dependent association with CPG2. *Proceedings of the National Academy of Sciences* 110(47), E4548-E4556. doi: 10.1073/pnas.1318860110.

Lomo, T. (2003). The discovery of long-term potentiation. *Philos Trans R Soc Lond B Biol Sci* 358(1432), 617-620. doi: 10.1098/rstb.2002.1226.

Lonergan, M.E., Gafford, G.M., Jarome, T.J., and Helmstetter, F.J. (2010). Time-dependent expression of Arc and zif268 after acquisition of fear conditioning. *Neural Plast* 2010, 139891. doi: 10.1155/2010/139891.

Lorenzen, A., Samosh, J., Vandewark, K., Anborgh, P.H., Seah, C., Magalhaes, A.C., et al. (2010). Rapid and Direct Transport of Cell Surface APP to the Lysosome defines a novel selective pathway. *Molecular Brain* 3(1), 11. doi: 10.1186/1756-6606-3-11.

Losonczy A, Zhang L, Shigemoto R, Somogyi P, Nusser Z (2002) Cell type dependence and variability in the short-term plasticity of EPSCs in identified mouse hippocampal interneurones. The Journal of physiology 542:193-210.

Lozada, A.F., Wang, X., Gounko, N.V., Massey, K.A., Duan, J., Liu, Z., et al. (2012). Glutamatergic Synapse Formation is Promoted by α7-Containing Nicotinic Acetylcholine Receptors. *The Journal of Neuroscience* 32(22), 7651-7661. doi: 10.1523/jneurosci.6246-11.2012.

Lu, J., Helton, T.D., Blanpied, T.A., Rácz, B., Newpher, T.M., Weinberg, R.J., et al. (2007a). Postsynaptic positioning of endocytic zones and AMPA receptor cycling by physical coupling of dynamin-3 to Homer. *Neuron* 55(6), 874-889. doi: 10.1016/j.neuron.2007.06.041.

Lu, W., and Roche, K.W. (2012). Posttranslational regulation of AMPA receptor trafficking and function. *Current opinion in neurobiology* 22(3), 470-479. doi: 10.1016/j.conb.2011.09.008.

Lu, W., Isozaki, K., Roche, K.W., and Nicoll, R.A. (2010). Synaptic targeting of AMPA receptors is regulated by a CaMKII site in the first intracellular loop of GluA1. *Proc Natl Acad Sci U S A* 107(51), 22266-22271. doi: 10.1073/pnas.1016289107.

Lu, W., Khatri, L., and Ziff, E.B. (2014). Trafficking of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPA) receptor subunit GluA2 from the endoplasmic reticulum is stimulated by a complex containing Ca^{2+}/calmodulin-activated kinase II (CaMKII) and PICK1 protein and by release of Ca^{2+} from internal stores. *Journal of Biological Chemistry* 289(27), 19218-19230.

Lu, W., Shi, Y., Jackson, A.C., Bjorgan, K., During, M.J., Sprengel, R., et al. (2009). Subunit composition of synaptic AMPA receptors revealed by a single-cell genetic approach. *Neuron* 62(2), 254-268. doi: 10.1016/j.neuron.2009.02.027.

Lu, Y., Allen, M., Halt, A.R., Weisenhaus, M., Dallapiazza, R.F., Hall, D.D., et al. (2007b). Age-dependent requirement of AKAP150-anchored PKA and GluR2-lacking AMPA receptors in LTP. *Embo j* 26(23), 4879-4890. doi: 10.1038/sj.emboj.7601884.

Lu, Y., Christian, K., and Lu, B. (2008). BDNF: a key regulator for protein synthesis-dependent LTP and long-term memory? *Neurobiology of learning and memory* 89(3), 312-323. doi: 10.1016/j.nlm.2007.08.018.

Lu, Y.F., and Hawkins, R.D. (2002). Ryanodine receptors contribute to cGMP-induced late-phase LTP and CREB phosphorylation in the hippocampus. *J Neurophysiol* 88(3), 1270-1278. doi: 10.1152/jn.2002.88.3.1270.

Luan, X., Sansanaphongpricha, K., Myers, I., Chen, H., Yuan, H., and Sun, D. (2017). Engineering exosomes as refined biological nanoplatforms for drug delivery. *Acta Pharmacologica Sinica* 38(6), 754-763. doi: 10.1038/aps.2017.12.

Lue, L.F., Kuo, Y.M., Roher, A.E., Brachova, L., Shen, Y., Sue, L., et al. (1999). Soluble amyloid beta peptide concentration as a predictor of synaptic change in Alzheimer's disease. *Am J Pathol* 155(3), 853-862. doi: 10.1016/s0002-9440(10)65184-x.

Lugaro, E. (1898). Le resistenze nell'evoluzione della vita. *Rivista moderna di cultura* 1, 29-60.

Luo, L., Chen, W.H., Wang, M., Zhu, D.M., She, J.Q., and Ruan, D.Y. (2008). Modulation of long-term potentiation by individual subtypes of muscarinic acetylcholine receptor in the rat dentate gyrus. *Hippocampus* 18(10), 989-995. doi: 10.1002/hipo.20461.

Lüscher, C., and Malenka, R.C. (2012). NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harbor perspectives in biology* 4(6), a005710. doi: 10.1101/cshperspect.a005710.

Lv, X.-F., Sun, L.-L., Cui, C.-L., and Han, J.-S. (2015). NAc Shell Arc/Arg3.1 Protein Mediates Reconsolidation of Morphine CPP by Increased GluR1 Cell Surface Expression: Activation of ERK-Coupled CREB is Required. *International Journal of Neuropsychopharmacology* 18(9). doi: 10.1093/ijnp/pyv030.

Lyford, G.L., Yamagata, K., Kaufmann, W.E., Barnes, C.A., Sanders, L.K., Copeland, N.G., et al. (1995). Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites. *Neuron* 14(2), 433-445.

Lynch, G., Browning, M., and Bennett, W.F. (1979). Biochemical and physiological studies of long-term synaptic plasticity. *Fed Proc* 38(7), 2117-2122.

Lynch, G., Larson, J., Kelso, S., Barrionuevo, G., and Schottler, F. (1983). Intracellular injections of EGTA block induction of hippocampal long-term potentiation. *Nature* 305(5936), 719-721.

Lynch, G.S., Dunwiddie, T., and Gribkoff, V. (1977). Heterosynaptic depression: a postsynaptic correlate of long-term potentiation. *Nature* 266(5604), 737-739.

Lynch, G.S., Gribkoff, V.K., and Deadwyler, S.A. (1976). Long term potentiation is accompanied by a reduction in dendritic responsiveness to glutamic acid. *Nature* 263, 151. doi: 10.1038/263151a0.

Lysetskiy, M., Földy, C., and Soltesz, I. (2005). Long- and short-term plasticity at mossy fiber synapses on mossy cells in the rat dentate gyrus. *Hippocampus* 15(6), 691-696. doi: 10.1002/hipo.20096.

Ma, H., Groth, R.D., Cohen, S.M., Emery, J.F., Li, B., Hoedt, E., et al. (2014a). γCaMKII shuttles Ca^{2+}/CaM to the nucleus to trigger CREB phosphorylation and gene expression. *Cell* 159(2), 281-294. doi: 10.1016/j.cell.2014.09.019.

Ma, K.-G., Lv, J., Yang, W.-N., Chang, K.-W., Hu, X.-D., Shi, L.-L., et al. (2018). The p38 mitogen activated protein kinase regulates β-amyloid protein internalization through the α7 nicotinic acetylcholine receptor in mouse brain. *Brain Research Bulletin* 137, 41-52. doi: https://doi.org/10.1016/j.brainresbull.2017.11.006.

Ma, Y.-Y., Wang, X., Huang, Y., Marie, H., Nestler, E.J., Schlüter, O.M., et al. (2016). Re-silencing of silent synapses unmasks anti-relapse effects of environmental enrichment. *Proceedings of the National Academy of Sciences of the United States of America* 113(18), 5089-5094. doi: 10.1073/pnas.1524739113.

Ma, Y.Y., Lee, B.R., Wang, X., Guo, C., Liu, L., Cui, R., et al. (2014b). Bidirectional modulation of incubation of cocaine craving by silent synapse-based remodeling of prefrontal cortex to accumbens projections. *Neuron* 83(6), 1453-1467. doi: 10.1016/j.neuron.2014.08.023.

Maccaferri G, Lacaille J-C (2003) Interneuron Diversity series: Hippocampal interneuron classifications; making things as simple as possible, not simpler. Trends in neurosciences 26:564-571.

Maccecchini, M.L., Chang, M.Y., Pan, C., John, V., Zetterberg, H., and Greig, N.H. (2012). Posiphen as a candidate drug to lower CSF amyloid precursor protein, amyloid-beta peptide and tau levels: target engagement, tolerability and pharmacokinetics in humans. *J Neurol Neurosurg Psychiatry* 83(9), 894-902. doi: 10.1136/jnnp-2012-302589.

MacDougall, M.J., and Fine, A. (2013). The expression of long-term potentiation: reconciling the preists and the postivists. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 369(1633), 20130135-20130135. doi: 10.1098/rstb.2013.0135.

Mack, V., Burnashev, N., Kaiser, K.M.M., Rozov, A., Jensen, V., Hvalby, Ø., et al. (2001). Conditional Restoration of Hippocampal Synaptic Potentiation in GluR-A-Deficient Mice. *Science* 292(5526), 2501-2504. doi: 10.1126/science.1059365.

Maeng, S., Zarate, C.A., Jr., Du, J., Schloesser, R.J., McCammon, J., Chen, G., et al. (2008). Cellular mechanisms underlying the antidepressant effects of ketamine: role of alpha-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors. *Biol Psychiatry* 63(4), 349-352. doi: 10.1016/j.biopsych.2007.05.028.

Mahajan, G., and Nadkarni, S. (2019). Intracellular calcium stores mediate metaplasticity at hippocampal dendritic spines. *The Journal of Physiology* 597(13), 3473-3502. doi: 10.1113/jp277726.

Mahanty, N.K., and Sah, P. (1998). Calcium-permeable AMPA receptors mediate long-term potentiation in interneurons in the amygdala. *Nature* 394(6694), 683-687. doi: 10.1038/29312.

Maheshwari, M., Samanta, A., Godavarthi, S.K., Mukherjee, R., and Jana, N.R. (2012). Dysfunction of the ubiquitin ligase Ube3a may be associated with synaptic pathophysiology in a mouse model

of Huntington disease. *The Journal of biological chemistry* 287(35), 29949-29957. doi: 10.1074/jbc.M112.371724.
Mahmmoud, R.R., Sase, S., Aher, Y.D., Sase, A., Gröger, M., Mokhtar, M., et al. (2015). Spatial and Working Memory Is Linked to Spine Density and Mushroom Spines. *PloS one* 10(10), e0139739-e0139739. doi: 10.1371/journal.pone.0139739.
Mahmoud, G.S., and Grover, L.M. (2006). Growth hormone enhances excitatory synaptic transmission in area CA1 of rat hippocampus. *J Neurophysiol* 95(5), 2962-2974. doi: 10.1152/jn.00947.2005.
Mainen, Z.F., Jia, Z., Roder, J., and Malinow, R. (1998). Use-dependent AMPA receptor block in mice lacking GluR2 suggests postsynaptic site for LTP expression. *Nat Neurosci* 1(7), 579-586. doi: 10.1038/2812.
Maity, S., Rah, S., Sonenberg, N., Gkogkas, C.G., and Nguyen, P.V. (2015). Norepinephrine triggers metaplasticity of LTP by increasing translation of specific mRNAs. *Learning & memory (Cold Spring Harbor, N.Y.)* 22(10), 499-508. doi: 10.1101/lm.039222.115.
Makino, H., and Malinow, R. (2009). AMPA Receptor Incorporation into Synapses during LTP: The Role of Lateral Movement and Exocytosis. *Neuron* 64(3), 381-390. doi: https://doi.org/10.1016/j.neuron.2009.08.035.
Makuch, L., Volk, L., Anggono, V., Johnson, R.C., Yu, Y., Duning, K., et al. (2011). Regulation of AMPA receptor function by the human memory-associated gene KIBRA. *Neuron* 71(6), 1022-1029. doi: 10.1016/j.neuron.2011.08.017.
Malenka, R.C. (1991a). Postsynaptic factors control the duration of synaptic enhancement in area CA1 of the hippocampus. *Neuron* 6(1), 53-60. doi: 10.1016/0896-6273(91)90121-f.
Malenka, R.C. (1991b). The role of postsynaptic calcium in the induction of long-term potentiation. *Mol Neurobiol* 5(2-4), 289-295.
Malenka, R.C., and Nicoll, R.A. (1999). Long-term potentiation--a decade of progress? *Science* 285(5435), 1870-1874.
Malenka, R.C., Kauer, J.A., Zucker, R.S., and Nicoll, R.A. (1988). Postsynaptic calcium is sufficient for potentiation of hippocampal synaptic transmission. *Science* 242(4875), 81-84.
Malinow, R., and Malenka, R.C. (2002). AMPA Receptor Trafficking and Synaptic Plasticity. *Annual Review of Neuroscience* 25(1), 103-126. doi: 10.1146/annurev.neuro.25.112701.142758.
Malinow, R., and Miller, J.P. (1986). Postsynaptic hyperpolarization during conditioning reversibly blocks induction of long-term potentiation. *Nature* 320(6062), 529-530. doi: 10.1038/320529a0.
Malinow, R., and Tsien, R.W. (1990). Presynaptic enhancement shown by whole-cell recordings of long-term potentiation in hippocampal slices. *Nature* 346(6280), 177-180. doi: 10.1038/346177a0.
Malinow, R., Schulman, H., and Tsien, R.W. (1989). Inhibition of postsynaptic PKC or CaMKII blocks induction but not expression of LTP. *Science* 245(4920), 862-866.
Mameli, M., Balland, B., Luján, R., and Lüscher, C. (2007). Rapid synthesis and synaptic insertion of GluR2 for mGluR-LTD in the ventral tegmental area. *Science* 317(5837), 530-533. doi: 10.1126/science.1142365.
Mammen, A.L., Kameyama, K., Roche, K.W., and Huganir, R.L. (1997). Phosphorylation of the alpha-amino-3-hydroxy-5-methylisoxazole4-propionic acid receptor GluR1 subunit by calcium/calmodulin-dependent kinase II. *J Biol Chem* 272(51), 32528-32533. doi: 10.1074/jbc.272.51.32528.
Man, H.-Y. (2011). GluA2-lacking, calcium-permeable AMPA receptors--inducers of plasticity? *Current opinion in neurobiology* 21(2), 291-298. doi: 10.1016/j.conb.2011.01.001.
Man, H.-Y., Lin, J.W., Ju, W.H., Ahmadian, G., Liu, L., Becker, L.E., et al. (2000). Regulation of AMPA Receptor-Mediated Synaptic Transmission by Clathrin-Dependent Receptor Internalization. *Neuron* 25(3), 649-662. doi: 10.1016/S0896-6273(00)81067-3.
Man, H.-Y., Sekine-Aizawa, Y., and Huganir, R.L. (2007). Regulation of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor trafficking through PKA phosphorylation of the Glu receptor 1

subunit. *Proceedings of the National Academy of Sciences* 104(9), 3579-3584. doi: 10.1073/pnas.0611698104.
Manahan-Vaughan, D., Kulla, A., and Frey, J.U. (2000). Requirement of translation but not transcription for the maintenance of long-term depression in the CA1 region of freely moving rats. *J Neurosci* 20(22), 8572-8576.
Mao, L.-M., Jin, D.-Z., Xue, B., Chu, X.-P., and Wang, J.Q. (2014). Phosphorylation and regulation of glutamate receptors by CaMKII. *Sheng li xue bao : [Acta physiologica Sinica]* 66(3), 365-372.
Mao, L., Takamiya, K., Thomas, G., Lin, D.-T., and Huganir, R.L. (2010). GRIP1 and 2 regulate activity-dependent AMPA receptor recycling via exocyst complex interactions. *Proceedings of the National Academy of Sciences* 107(44), 19038-19043. doi: 10.1073/pnas.1013494107.
Marcade, M., Bourdin, J., Loiseau, N., Peillon, H., Rayer, A., Drouin, D., et al. (2008). Etazolate, a neuroprotective drug linking GABA(A) receptor pharmacology to amyloid precursor protein processing. *J Neurochem* 106(1), 392-404. doi: 10.1111/j.1471-4159.2008.05396.x.
Maroteaux, M., Valjent, E., Longueville, S., Topilko, P., Girault, J.-A., and Hervé, D. (2014). Role of the Plasticity-Associated Transcription Factor Zif268 in the Early Phase of Instrumental Learning. *PLOS ONE* 9(1), e81868. doi: 10.1371/journal.pone.0081868.
Marslen-Wilson, W.D., and Teuber, H.L. (1975). Memory for remote events in anterograde amnesia: recognition of public figures from newsphotographs. *Neuropsychologia* 13(3), 353-364.
Martenyi, F., Dean, R.A., Lowe, S., Nakano, M., Monk, S., Willis, B.A., et al. (2012). P3-363: BACE inhibitor LY2886721 safety and central and peripheral PK and PD in healthy subjects (HSs). *Alzheimer's & Dementia* 8(4S_Part_16), P583-P584. doi: 10.1016/j.jalz.2012.05.1588.
Martin, A., Stillman, J., Miguez, M.-J., McDaniel, H.R., Konefal, J., Woolger, J.M., et al. (2017). The effect of dietary supplementation on brain-derived neurotrophic factor and cognitive functioning in Alzheimer's dementia. *Journal of clinical and translational research* 3(3), 337-343.
Martin, F.H., Suggs, S.V., Langley, K.E., Lu, H.S., Ting, J., Okino, K.H., et al. (1990). Primary structure and functional expression of rat and human stem cell factor DNAs. *Cell* 63(1), 203-211. doi: 10.1016/0092-8674(90)90301-t.
Martinsson, I., Capetillo-Zarate, E., Faideau, M., Willén, K., Esteras, N., Frykman, S., et al. (2019). APP depletion alters selective pre- and post-synaptic proteins. *Molecular and Cellular Neuroscience* 95, 86-95. doi: https://doi.org/10.1016/j.mcn.2019.02.003.
Masters, C.L., Simms, G., Weinman, N.A., Multhaup, G., McDonald, B.L., and Beyreuther, K. (1985). Amyloid plaque core protein in Alzheimer disease and Down syndrome. *Proc Natl Acad Sci U S A* 82(12), 4245-4249.
Maswood, N., Young, J., Tilmont, E., Zhang, Z., Gash, D.M., Gerhardt, G.A., et al. (2004). Caloric restriction increases neurotrophic factor levels and attenuates neurochemical and behavioral deficits in a primate model of Parkinson's disease. *Proc Natl Acad Sci U S A* 101(52), 18171-18176. doi: 10.1073/pnas.0405831102.
Matsuda, S., Kakegawa, W., Budisantoso, T., Nomura, T., Kohda, K., and Yuzaki, M. (2013). Stargazin regulates AMPA receptor trafficking through adaptor protein complexes during long-term depression. *Nature Communications* 4(1), 2759. doi: 10.1038/ncomms3759.
Matsuda, S., Launey, T., Mikawa, S., and Hirai, H. (2000). Disruption of AMPA receptor GluR2 clusters following long-term depression induction in cerebellar Purkinje neurons. *Embo j* 19(12), 2765-2774. doi: 10.1093/emboj/19.12.2765.
Matsuzaki, M., Ellis-Davies, G.C.R., Nemoto, T., Miyashita, Y., Iino, M., and Kasai, H. (2001). Dendritic spine geometry is critical for AMPA receptor expression in hippocampal CA1 pyramidal neurons. *Nature Neuroscience* 4(11), 1086-1092. doi: 10.1038/nn736.
Matsuzaki, M., Honkura, N., Ellis-Davies, G.C., and Kasai, H. (2004a). Structural basis of long-term potentiation in single dendritic spines. *Nature* 429(6993), 761-766. doi: 10.1038/nature02617.
Matthias, K., Kirchhoff, F., Seifert, G., Huttmann, K., Matyash, M., Kettenmann, H., et al. (2003). Segregated expression of AMPA-type glutamate receptors and glutamate transporters defines distinct astrocyte populations in the mouse hippocampus. *J Neurosci* 23(5), 1750-1758.

Mattison, H.A., Bagal, A.A., Mohammadi, M., Pulimood, N.S., Reich, C.G., Alger, B.E., et al. (2014). Evidence of calcium-permeable AMPA receptors in dendritic spines of CA1 pyramidal neurons. *J Neurophysiol* 112(2), 263-275. doi: 10.1152/jn.00578.2013.

Mattson, M.P., Cheng, B., Culwell, A.R., Esch, F.S., Lieberburg, I., and Rydel, R.E. (1993). Evidence for excitoprotective and intraneuronal calcium-regulating roles for secreted forms of the beta-amyloid precursor protein. *Neuron* 10(2), 243-254.

Matynia, A., Kushner, S.A., and Silva, A.J. (2002). Genetic approaches to molecular and cellular cognition: a focus on LTP and learning and memory. *Annu Rev Genet* 36, 687-720. doi: 10.1146/annurev.genet.36.062802.091007.

Mauch, D.H., Nägler, K., Schumacher, S., Göritz, C., Müller, E.C., Otto, A., et al. (2001). CNS synaptogenesis promoted by glia-derived cholesterol. *Science* 294(5545), 1354-1357. doi: 10.1126/science.294.5545.1354.

Maximov, A., Pang, Z.P., Tervo, D.G., and Südhof, T.C. (2007). Monitoring synaptic transmission in primary neuronal cultures using local extracellular stimulation. *J Neurosci Methods* 161(1), 75-87. doi: 10.1016/j.jneumeth.2006.10.009.

Mayer, M.L. (2005). Glutamate receptor ion channels. *Curr Opin Neurobiol* 15(3), 282-288. doi: 10.1016/j.conb.2005.05.004.

Maynard, C.J., Cappai, R., Volitakis, I., Cherny, R.A., White, A.R., Beyreuther, K., et al. (2002). Overexpression of Alzheimer's disease amyloid-beta opposes the age-dependent elevations of brain copper and iron. *J Biol Chem* 277(47), 44670-44676. doi: 10.1074/jbc.M204379200.

McAllister, A.K., Katz, L.C., and Lo, D.C. (1999). NEUROTROPHINS AND SYNAPTIC PLASTICITY. *Annual Review of Neuroscience* 22(1), 295-318. doi: 10.1146/annurev.neuro.22.1.295.

McBain, C.J., and Mayer, M.L. (1994). N-methyl-D-aspartic acid receptor structure and function. *Physiol Rev* 74(3), 723-760.

McCarthy, J.B., and Milner, T.A. (2003). Dendritic ribosomes suggest local protein synthesis during estrous synaptogenesis. *Neuroreport* 14(10), 1357-1360. doi: 10.1097/01.wnr.0000078380.40088.99.

McClelland, J.L., McNaughton, B.L., and O'Reilly, R.C. (1995). Why there are complementary learning systems in the hippocampus and neocortex: insights from the successes and failures of connectionist models of learning and memory. *Psychol Rev* 102(3), 419-457. doi: 10.1037/0033-295x.102.3.419.

McCormack, S.G., Stornetta, R.L., and Zhu, J.J. (2006). Synaptic AMPA receptor exchange maintains bidirectional plasticity. *Neuron* 50(1), 75-88. doi: 10.1016/j.neuron.2006.02.027.

McCutcheon, J.E., Loweth, J.A., Ford, K.A., Marinelli, M., Wolf, M.E., and Tseng, K.Y. (2011). Group I mGluR activation reverses cocaine-induced accumulation of calcium-permeable AMPA receptors in nucleus accumbens synapses via a protein kinase C-dependent mechanism. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 31(41), 14536-14541. doi: 10.1523/JNEUROSCI.3625-11.2011.

McGehee, D.S. (1999). Molecular Diversity of Neuronal Nicotinic Acetylcholine Receptors. *Annals of the New York Academy of Sciences* 868(1), 565-577. doi: 10.1111/j.1749-6632.1999.tb11330.x.

McKinney, R.A., Capogna, M., Dürr, R., Gähwiler, B.H., Thompson, and Scott, M. (1999). Miniature synaptic events maintain dendritic spines via AMPA receptor activation. *Nature Neuroscience* 2(1), 44-49. doi: 10.1038/4548.

Meakin, P.J., Jalicy, S.M., Montagut, G., Allsop, D.J.P., Cavellini, D.L., Irvine, S.W., et al. (2018). Bace1-dependent amyloid processing regulates hypothalamic leptin sensitivity in obese mice. *Scientific Reports* 8(1), 55. doi: 10.1038/s41598-017-18388-6.

Medina, J.M., and Tabernero, A. (2002). Astrocyte-synthesized oleic acid behaves as a neurotrophic factor for neurons. *J Physiol Paris* 96(3-4), 265-271. doi: 10.1016/s0928-4257(02)00015-3.

Mei, F., Nagappan, G., Ke, Y., Sacktor, T.C., and Lu, B. (2011). BDNF Facilitates L-LTP Maintenance in the Absence of Protein Synthesis through PKMζ. *PLOS ONE* 6(6), e21568. doi: 10.1371/journal.pone.0021568.

Meng, Y., Zhang, Y., and Jia, Z. (2003). Synaptic Transmission and Plasticity in the Absence of AMPA Glutamate Receptor GluR2 and GluR3. *Neuron* 39(1), 163-176. doi: https://doi.org/10.1016/S0896-6273(03)00368-4.

Messaoudi, E., Kanhema, T., Soulé, J., Tiron, A., Dagyte, G., da Silva, B., et al. (2007). Sustained Arc/Arg3.1 Synthesis Controls Long-Term Potentiation Consolidation through Regulation of Local Actin Polymerization in the Dentate Gyrus In Vivo. *The Journal of Neuroscience* 27(39), 10445-10455. doi: 10.1523/jneurosci.2883-07.2007.

Messaoudi, E., Ying, S.-W., Kanhema, T., Croll, S.D., and Bramham, C.R. (2002). Brain-derived neurotrophic factor triggers transcription-dependent, late phase long-term potentiation in vivo. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 22(17), 7453-7461. doi: 10.1523/JNEUROSCI.22-17-07453.2002.

Meziane, H., Dodart, J.C., Mathis, C., Little, S., Clemens, J., Paul, S.M., et al. (1998). Memory-enhancing effects of secreted forms of the beta-amyloid precursor protein in normal and amnestic mice. *Proc Natl Acad Sci U S A* 95(21), 12683-12688. doi: 10.1073/pnas.95.21.12683.

Michel, J.J., and Scott, J.D. (2002). AKAP mediated signal transduction. *Annu Rev Pharmacol Toxicol* 42, 235-257. doi: 10.1146/annurev.pharmtox.42.083101.135801.

Michel, M., Green, C.L., and Lyons, L.C. (2011). PKA and PKC are required for long-term but not short-term in vivo operant memory in Aplysia. *Learn Mem* 18(1), 19-23. doi: 10.1101/lm.2026311.

Michel, M., Green, C.L., Eskin, A., and Lyons, L.C. (2011). PKG-mediated MAPK signaling is necessary for long-term operant memory in Aplysia. *Learn Mem* 18(2), 108-117. doi: 10.1101/lm.2063611.

Migues, P.V., Hardt, O., Wu, D.C., Gamache, K., Sacktor, T.C., Wang, Y.T., et al. (2010). PKMzeta maintains memories by regulating GluR2-dependent AMPA receptor trafficking. *Nat Neurosci* 13(5), 630-634. doi: 10.1038/nn.2531.

Mikati, M.A., Grintsevich, E.E., and Reisler, E. (2013). Drebrin-induced Stabilization of Actin Filaments. *Journal of Biological Chemistry* 288(27), 19926-19938. doi: 10.1074/jbc.M113.472647.

Mileusnic, R., Lancashire, C.L., and Rose, S.P. (2004). The peptide sequence Arg-Glu-Arg, present in the amyloid precursor protein, protects against memory loss caused by A beta and acts as a cognitive enhancer. *Eur J Neurosci* 19(7), 1933-1938. doi: 10.1111/j.1460-9568.2004.03276.x.

Mileusnic, R., Lancashire, C.L., Johnston, A.N., and Rose, S.P. (2000). APP is required during an early phase of memory formation. *Eur J Neurosci* 12(12), 4487-4495.

Millet, L.J., and Gillette, M.U. (2012). Over a century of neuron culture: from the hanging drop to microfluidic devices. *Yale J Biol Med* 85(4), 501-521.

Milosch, N., Tanriover, G., Kundu, A., Rami, A., Francois, J.C., Baumkotter, F., et al. (2014a). Holo-APP and G-protein-mediated signaling are required for sAPPalpha-induced activation of the Akt survival pathway. *Cell Death Dis* 5, e1391. doi: 10.1038/cddis.2014.352.

Milosch, N., Tanriöver, G., Kundu, A., Rami, A., François, J.C., Baumkötter, F., et al. (2014b). Holo-APP and G-protein-mediated signaling are required for sAPPα-induced activation of the Akt survival pathway. *Cell Death & Disease* 5(8), e1391-e1391. doi: 10.1038/cddis.2014.352.

Minatohara, K., Akiyoshi, M., and Okuno, H. (2016). Role of Immediate-Early Genes in Synaptic Plasticity and Neuronal Ensembles Underlying the Memory Trace. *Frontiers in molecular neuroscience* 8, 78-78. doi: 10.3389/fnmol.2015.00078.

Minichiello, L., Korte, M., Wolfer, D., Kühn, R., Unsicker, K., Cestari, V., et al. (1999). Essential role for TrkB receptors in hippocampus-mediated learning. *Neuron* 24(2), 401-414. doi: 10.1016/s0896-6273(00)80853-3.

Mitsushima, D., Sano, A., and Takahashi, T. (2013). A cholinergic trigger drives learning-induced plasticity at hippocampal synapses. *Nature Communications* 4(1), 2760. doi: 10.1038/ncomms3760.

Miyajima, M., Nakajima, M., Ogino, I., Miyata, H., Motoi, Y., and Arai, H. (2013). Soluble amyloid precursor protein alpha in the cerebrospinal fluid as a diagnostic and prognostic biomarker for idiopathic normal pressure hydrocephalus. *Eur J Neurol* 20(2), 236-242. doi: 10.1111/j.1468-1331.2012.03781.x.

Mizui, T., Takahashi, H., Sekino, Y., and Shirao, T. (2005). Overexpression of drebrin A in immature neurons induces the accumulation of F-actin and PSD-95 into dendritic filopodia, and the formation of large abnormal protrusions. *Mol Cell Neurosci* 30(1), 149-157. doi: 10.1016/j.mcn.2005.06.008.

Mlinar, B., Stocca, G., and Corradetti, R. (2015a). Endogenous serotonin facilitates hippocampal long-term potentiation at CA3/CA1 synapses. *Journal of Neural Transmission* 122(2), 177-185. doi: 10.1007/s00702-014-1246-7.

Mocellin, S., and Provenzano, M. (2004). RNA interference: learning gene knock-down from cell physiology. *Journal of translational medicine* 2(1), 39-39. doi: 10.1186/1479-5876-2-39.

Mockett, B.G., Guévremont, D., Elder, M.K., Parfitt, K.D., Peppercorn, K., Morrissey, J., et al. (2019). Glutamate Receptor Trafficking and Protein Synthesis Mediate the Facilitation of LTP by Secreted Amyloid Precursor Protein-Alpha. *The Journal of Neuroscience* 39(17), 3188-3203. doi: 10.1523/jneurosci.1826-18.2019.

Mockett, B.G., Guevremont, D., Wutte, M., Hulme, S.R., Williams, J.M., and Abraham, W.C. (2011). Calcium/calmodulin-dependent protein kinase II mediates group I metabotropic glutamate receptor-dependent protein synthesis and long-term depression in rat hippocampus. *J Neurosci* 31(20), 7380-7391. doi: 10.1523/jneurosci.6656-10.2011.

Moga, D.E., Calhoun, M.E., Chowdhury, A., Worley, P., Morrison, J.H., and Shapiro, M.L. (2004). Activity-regulated cytoskeletal-associated protein is localized to recently activated excitatory synapses. *Neuroscience* 125(1), 7-11. doi: 10.1016/j.neuroscience.2004.02.004.

Mokin, M., and Keifer, J. (2004). Targeting of GLUR4-containing AMPA receptors to synaptic sites during in vitro classical conditioning. *Neuroscience* 128(2), 219-228. doi: https://doi.org/10.1016/j.neuroscience.2004.06.009.

Mokin, M., Lindahl, J.S., and Keifer, J. (2006). Immediate-Early Gene-Encoded Protein Arc Is Associated With Synaptic Delivery of GluR4-containing AMPA Receptors During In Vitro Classical Conditioning. *Journal of Neurophysiology* 95(1), 215-224. doi: 10.1152/jn.00737.2005.

Mokin, M., Zheng, Z., and Keifer, J. (2007). Conversion of Silent Synapses Into the Active Pool by Selective GluR1-3 and GluR4 AMPAR Trafficking During In Vitro Classical Conditioning. *Journal of Neurophysiology* 98(3), 1278-1286. doi: 10.1152/jn.00212.2007.

Molina, D.P., Ariwodola, O.J., Linville, C., Sonntag, W.E., Weiner, J.L., Brunso-Bechtold, J.K., et al. (2012). Growth hormone modulates hippocampal excitatory synaptic transmission and plasticity in old rats. *Neurobiology of aging* 33(9), 1938-1949. doi: 10.1016/j.neurobiolaging.2011.09.014.

Mollá, B., Muñoz-Lasso, D.C., Riveiro, F., Bolinches-Amorós, A., Pallardó, F.V., Fernandez-Vilata, A., et al. (2017). Reversible Axonal Dystrophy by Calcium Modulation in Frataxin-Deficient Sensory Neurons of YG8R Mice. *Frontiers in Molecular Neuroscience* 10(264). doi: 10.3389/fnmol.2017.00264.

Monfort, P., Munoz, M.D., Kosenko, E., Llansola, M., Sanchez-Perez, A., Cauli, O., et al. (2004). Sequential activation of soluble guanylate cyclase, protein kinase G and cGMP-degrading phosphodiesterase is necessary for proper induction of long-term potentiation in CA1 of hippocampus. Alterations in hyperammonemia. *Neurochem Int* 45(6), 895-901. doi: 10.1016/j.neuint.2004.03.020.

Montgomery, J.M., Pavlidis, P., and Madison, D.V. (2001). Pair recordings reveal all-silent synaptic connections and the postsynaptic expression of long-term potentiation. *Neuron* 29(3), 691-701. doi: 10.1016/s0896-6273(01)00244-6.

Moreno, L., Rose, C., Mohanraj, A., Allinquant, B., Billard, J.M., and Dutar, P. (2015). sAbetaPPalpha Improves Hippocampal NMDA-Dependent Functional Alterations Linked to Healthy Aging. *J Alzheimers Dis* 48(4), 927-935. doi: 10.3233/jad-150297.

Morise, J., Suzuki, K.G.N., Kitagawa, A., Wakazono, Y., Takamiya, K., Tsunoyama, T.A., et al. (2019). AMPA receptors in the synapse turnover by monomer diffusion. *Nature Communications* 10(1), 5245. doi: 10.1038/s41467-019-13229-8.

Morita, D., Rah, J.C., and Isaac, J.T.R. (2013). Incorporation of inwardly rectifying AMPA receptors at silent synapses during hippocampal long-term potentiation. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 369(1633), 20130156-20130156. doi: 10.1098/rstb.2013.0156.

Mørkve, S.H., Veruki, M.L., and Hartveit, E. (2002). Functional characteristics of non-NMDA-type ionotropic glutamate receptor channels in all amacrine cells in rat retina. *Journal of Physiology* 542(1), 147-165. doi: 10.1113/jphysiol.2002.020305.

Morley, J.E., and Farr, S.A. (2016). Alzheimer Mythology: A Time to Think Out of the Box. *J Am Med Dir Assoc* 17(9), 769-774. doi: 10.1016/j.jamda.2016.06.017.

Morris, R.G., Anderson, E., Lynch, G.S., and Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an N-methyl-D-aspartate receptor antagonist, AP5. *Nature* 319(6056), 774-776. doi: 10.1038/319774a0.

Morrissey, J.A., Bigus, E., Necarsulmer, J.C., Srinivasan, V., Peppercorn, K., O'Leary, D.J., et al. (2019a). The Tripeptide RER Mimics Secreted Amyloid Precursor Protein-Alpha in Upregulating LTP. *Frontiers in cellular neuroscience* 13, 459-459. doi: 10.3389/fncel.2019.00459.

Morrissey, J.A., Mockett, B.G., Singh, A., Kweon, D., Ohline, S.M., Tate, W.P., et al. (2019b). A C-terminal peptide from secreted amyloid precursor protein-α enhances long-term potentiation in rats and a transgenic mouse model of Alzheimer's disease. *Neuropharmacology* 157, 107670. doi: 10.1016/j.neuropharm.2019.107670.

Moult, P.R., and Harvey, J. (2008). Hormonal regulation of hippocampal dendritic morphology and synaptic plasticity. *Cell adhesion & migration* 2(4), 269-275. doi: 10.4161/cam.2.4.6354.

Mousavi, M., and Hellstrom-Lindahl, E. (2009). Nicotinic receptor agonists and antagonists increase sAPPalpha secretion and decrease Abeta levels in vitro. *Neurochem Int* 54(3-4), 237-244. doi: 10.1016/j.neuint.2008.12.001.

Moussawi, K., Pacchioni, A., Moran, M., Olive, M.F., Gass, J.T., Lavin, A., et al. (2009). N-Acetylcysteine reverses cocaine-induced metaplasticity. *Nat Neurosci* 12(2), 182-189. doi: 10.1038/nn.2250.

Mucke, L., Abraham, C.R., and Masliah, E. (1996). Neurotrophic and neuroprotective effects of hAPP in transgenic mice. *Ann N Y Acad Sci* 777, 82-88. doi: 10.1111/j.1749-6632.1996.tb34405.x.

Mulkey, R., Herron, C., and Malenka, R. (1993). An essential role for protein phosphatases in hippocampal long-term depression. *Science* 261(5124), 1051-1055. doi: 10.1126/science.8394601.

Mulkey, R.M., and Malenka, R.C. (1992). Mechanisms underlying induction of homosynaptic long-term depression in area CA1 of the hippocampus. *Neuron* 9(5), 967-975. doi: https://doi.org/10.1016/0896-6273(92)90248-C.

Müller C, Remy S (2014) Dendritic inhibition mediated by O-LM and bistratified interneurons in the hippocampus. Frontiers in Synaptic Neuroscience 6.

Müller, A., Stellmacher, A., Freitag, C.E., Landgraf, P., and Dieterich, D.C. (2015). Monitoring Astrocytic Proteome Dynamics by Cell Type-Specific Protein Labeling. *PLOS ONE* 10(12), e0145451. doi: 10.1371/journal.pone.0145451.

Muller, D., Buchs, P.A., Dunant, Y., and Lynch, G. (1990). Protein kinase C activity is not responsible for the expression of long-term potentiation in hippocampus. *Proc Natl Acad Sci U S A* 87(11), 4073-4077.

Muller, D., Hefft, S., and Figurov, A. (1995). Heterosynaptic interactions between LTP and LTD in CA1 hippocampal slices. *Neuron* 14(3), 599-605.

Müller, T., Meyer, H.E., Egensperger, R., and Marcus, K. (2008). The amyloid precursor protein intracellular domain (AICD) as modulator of gene expression, apoptosis, and cytoskeletal dynamics—Relevance for Alzheimer's disease. *Progress in Neurobiology* 85(4), 393-406. doi: https://doi.org/10.1016/j.pneurobio.2008.05.002.

Muller, T., Moller, T., Berger, T., Schnitzer, J., and Kettenmann, H. (1992). Calcium entry through kainate receptors and resulting potassium-channel blockade in Bergmann glial cells. *Science* 256(5063), 1563-1566.

Müller, U., Cristina, N., Li, Z.W., Wolfer, D.P., Lipp, H.P., Rülicke, T., et al. (1994). Behavioral and anatomical deficits in mice homozygous for a modified beta-amyloid precursor protein gene. *Cell* 79(5), 755-765. doi: 10.1016/0092-8674(94)90066-3.

Müller, W., and Connor, J.A. (1991). Dendritic spines as individual neuronal compartments for synaptic Ca^{2+} responses. *Nature* 354, 73. doi: 10.1038/354073a0.

Murthy, V.N., Schikorski, T., Stevens, C.F., and Zhu, Y. (2001). Inactivity produces increases in neurotransmitter release and synapse size. *Neuron* 32(4), 673-682.

Myrum, C., Soulé, J., Bittins, M., Cavagnini, K., Goff, K., Ziemek, S.K., et al. (2017). Arc Interacts with the Integral Endoplasmic Reticulum Protein, Calnexin. *Frontiers in Cellular Neuroscience* 11(294). doi: 10.3389/fncel.2017.00294.

Na, Y., Park, S., Lee, C., Kim, D.-K., Park, J.M., Sockanathan, S., et al. (2016). Real-Time Imaging Reveals Properties of Glutamate-Induced Arc/Arg 3.1 Translation in Neuronal Dendrites. *Neuron* 91(3), 561-573. doi: https://doi.org/10.1016/j.neuron.2016.06.017.

Nabavi, S., Fox, R., Proulx, C.D., Lin, J.Y., Tsien, R.Y., and Malinow, R. (2014). Engineering a memory with LTD and LTP. *Nature* 511, 348. doi: 10.1038/nature13294.

Nägerl, U.V., Eberhorn, N., Cambridge, S.B., and Bonhoeffer, T. (2004). Bidirectional Activity-Dependent Morphological Plasticity in Hippocampal Neurons. *Neuron* 44(5), 759-767. doi: 10.1016/j.neuron.2004.11.016.

Nair, D., Hosy, E., Petersen, J.D., Constals, A., Giannone, G., Choquet, D., et al. (2013). Super-resolution imaging reveals that AMPA receptors inside synapses are dynamically organized in nanodomains regulated by PSD95. *J Neurosci* 33(32), 13204-13224. doi: 10.1523/jneurosci.2381-12.2013.

Nair, R.R., Patil, S., Tiron, A., Kanhema, T., Panja, D., Schiro, L., et al. (2017). Dynamic Arc SUMOylation and Selective Interaction with F-Actin-Binding Protein Drebrin A in LTP Consolidation In Vivo. *Frontiers in synaptic neuroscience* 9, 8-8. doi: 10.3389/fnsyn.2017.00008.

Nakata, H., and Nakamura, S. (2007). Brain-derived neurotrophic factor regulates AMPA receptor trafficking to post-synaptic densities via IP3R and TRPC calcium signaling. *FEBS Lett* 581(10), 2047-2054. doi: 10.1016/j.febslet.2007.04.041.

Nakayama, K., Kiyosue, K., and Taguchi, T. (2005). Diminished neuronal activity increases neuron-neuron connectivity underlying silent synapse formation and the rapid conversion of silent to functional synapses. *J Neurosci* 25(16), 4040-4051. doi: 10.1523/jneurosci.4115-04.2005.

Nalbantoglu, J., Tirado-Santiago, G., Lahsaïni, A., Poirier, J., Goncalves, O., Verge, G., et al. (1997). Impaired learning and LTP in mice expressing the carboxy terminus of the Alzheimer amyloid precursor protein. *Nature* 387(6632), 500-505. doi: 10.1038/387500a0.

Narisawa-Saito, M., Silva, A.J., Yamaguchi, T., Hayashi, T., Yamamoto, T., and Nawa, H. (1999). Growth factor-mediated Fyn signaling regulates α-amino-3- hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor expression in rodent neocortical neurons. *Proceedings of the National Academy of Sciences* 96(5), 2461-2466. doi: 10.1073/pnas.96.5.2461.

Nayak, A., Zastrow, D.J., Lickteig, R., Zahniser, N.R., and Browning, M.D. (1998). Maintenance of late-phase LTP is accompanied by PKA-dependent increase in AMPA receptor synthesis. *Nature* 394, 680. doi: 10.1038/29305.

Needham, B.E., Ciccotosto, G.D., and Cappai, R. (2014). Combined deletions of amyloid precursor protein and amyloid precursor-like protein 2 reveal different effects on mouse brain metal homeostasis. *Metallomics* 6(3), 598-603. doi: 10.1039/c3mt00358b.

Needham, B.E., Wlodek, M.E., Ciccotosto, G.D., Fam, B.C., Masters, C.L., Proietto, J., et al. (2008). Identification of the Alzheimer's disease amyloid precursor protein (APP) and its homologue APLP2 as essential modulators of glucose and insulin homeostasis and growth. *J Pathol* 215(2), 155-163. doi: 10.1002/path.2343.

Neher, E., and Sakaba, T. (2008). Multiple roles of calcium ions in the regulation of neurotransmitter release. *Neuron* 59(6), 861-872. doi: 10.1016/j.neuron.2008.08.019.

Nery, A.A., Magdesian, M.H., Trujillo, C.A., Sathler, L.B., Juliano, M.A., Juliano, L., et al. (2013). Rescue of Amyloid-Beta-Induced Inhibition of Nicotinic Acetylcholine Receptors by a Peptide Homologous to the Nicotine Binding Domain of the Alpha 7 Subtype. *PLOS ONE* 8(7), e67194. doi: 10.1371/journal.pone.0067194.

Nguyen, P.V., Abel, T., and Kandel, E.R. (1994). Requirement of a critical period of transcription for induction of a late phase of LTP. *Science* 265(5175), 1104-1107.

Nicoll, R.A., Tomita, S., and Bredt, D.S. (2006). Auxiliary Subunits Assist AMPA-Type Glutamate Receptors. *Science* 311(5765), 1253-1256. doi: 10.1126/science.1123339.

Niethammer, M., Kim, E., and Sheng, M. (1996). Interaction between the C terminus of NMDA receptor subunits and multiple members of the PSD-95 family of membrane-associated guanylate kinases. *The Journal of Neuroscience* 16(7), 2157-2163. doi: 10.1523/jneurosci.16-07-02157.1996.

Nikolaienko, O., Patil, S., Eriksen, M.S., and Bramham, C.R. (2018). Arc protein: a flexible hub for synaptic plasticity and cognition. *Seminars in Cell & Developmental Biology* 77, 33-42. doi: https://doi.org/10.1016/j.semcdb.2017.09.006.

Nimchinsky, E.A., Sabatini, B.L., and Svoboda, K. (2002). Structure and function of dendritic spines. *Annu Rev Physiol* 64, 313-353. doi: 10.1146/annurev.physiol.64.081501.160008.

Ninomiya, H., Roch, J.M., Jin, L.W., and Saitoh, T. (1994). Secreted form of amyloid beta/A4 protein precursor (APP) binds to two distinct APP binding sites on rat B103 neuron-like cells through two different domains, but only one site is involved in neuritotropic activity. *J Neurochem* 63(2), 495-500. doi: 10.1046/j.1471-4159.1994.63020495.x.

Nishimune, A., Isaac, J.T.R., Molnar, E., Noel, J., Nash, S.R., Tagaya, M., et al. (1998). NSF Binding to GluR2 Regulates Synaptic Transmission. *Neuron* 21(1), 87-97. doi: 10.1016/S0896-6273(00)80517-6.

Nitsch, R., Slack, B., Wurtman, R., and Growdon, J. (1992). Release of Alzheimer amyloid precursor derivatives stimulated by activation of muscarinic acetylcholine receptors. *Science* 258(5080), 304-307. doi: 10.1126/science.1411529.

Nitsch, R.M., Farber, S.A., Growdon, J.H., and Wurtman, R.J. (1993). Release of amyloid beta-protein precursor derivatives by electrical depolarization of rat hippocampal slices. *Proc Natl Acad Sci U S A* 90(11), 5191-5193.

Nitsch, R.M., Wurtman, R.J., and Growdon, J.H. (1996). Regulation of APP processing. Potential for the therapeutical reduction of brain amyloid burden. *Ann N Y Acad Sci* 777, 175-182. doi: 10.1111/j.1749-6632.1996.tb34416.x.

Noorbala, A.A., Akhondzadeh, S., Davari-Ashtiani, R., and Amini-Nooshabadi, H. (1999). Piracetam in the treatment of schizophrenia: implications for the glutamate hypothesis of schizophrenia. *J Clin Pharm Ther* 24(5), 369-374. doi: 10.1046/j.1365-2710.1999.00238.x.

Nowak, L., Bregestovski, P., Ascher, P., Herbet, A., and Prochiantz, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* 307(5950), 462-465.

O'Brien, R.J., and Wong, P.C. (2011). Amyloid precursor protein processing and Alzheimer's disease. *Annu Rev Neurosci* 34, 185-204. doi: 10.1146/annurev-neuro-061010-113613.

O'Mara, S.M., Commins, S., Anderson, M., and Gigg, J. (2001). The subiculum: a review of form, physiology and function. *Prog Neurobiol* 64(2), 129-155. doi: 10.1016/s0301-0082(00)00054-x.

Obregon, D., Hou, H., Deng, J., Giunta, B., Tian, J., Darlington, D., et al. (2012). Soluble amyloid precursor protein-alpha modulates beta-secretase activity and amyloid-beta generation. *Nat Commun* 3, 777. doi: 10.1038/ncomms1781.

Obregon, D.F., Rezai-Zadeh, K., Bai, Y., Sun, N., Hou, H., Ehrhart, J., et al. (2006). ADAM10 activation is required for green tea (-)-epigallocatechin-3-gallate-induced alpha-secretase cleavage of amyloid precursor protein. *J Biol Chem* 281(24), 16419-16427. doi: 10.1074/jbc.M600617200.

Oh, J.S., Manzerra, P., and Kennedy, M.B. (2004). Regulation of the Neuron-specific Ras GTPase-activating Protein, synGAP, by Ca^{2+}/Calmodulin-dependent Protein Kinase II. *Journal of Biological Chemistry* 279(17), 17980-17988. doi: 10.1074/jbc.M314109200.

Oh, M.C., Derkach, V.A., Guire, E.S., and Soderling, T.R. (2006). Extrasynaptic Membrane Trafficking Regulated by GluR1 Serine 845 Phosphorylation Primes AMPA Receptors for Long-term Potentiation. *Journal of Biological Chemistry* 281(2), 752-758. doi: 10.1074/jbc.M509677200.

Okano, H. (2000). Learning and memory. 97(23), 12403-12404.

Okuno, H., Akashi, K., Ishii, Y., Yagishita-Kyo, N., Suzuki, K., Nonaka, M., et al. (2012). Inverse synaptic tagging of inactive synapses via dynamic interaction of Arc/Arg3.1 with CaMKIIβ. *Cell* 149(4), 886-898. doi: 10.1016/j.cell.2012.02.062.

Olayioye, M.A., Barisic, S., and Hausser, A. (2013). Multi-level control of actin dynamics by protein kinase D. *Cell Signal* 25(9), 1739-1747. doi: 10.1016/j.cellsig.2013.04.010.

Olsson, A., Höglund, K., Sjögren, M., Andreasen, N., Minthon, L., Lannfelt, L., et al. (2003). Measurement of alpha- and beta-secretase cleaved amyloid precursor protein in cerebrospinal fluid from Alzheimer patients. *Exp Neurol* 183(1), 74-80. doi: 10.1016/s0014-4886(03)00027-x.

Opazo, P., Labrecque, S., Tigaret, C.M., Frouin, A., Wiseman, P.W., De Koninck, P., et al. (2010). CaMKII triggers the diffusional trapping of surface AMPARs through phosphorylation of stargazin. *Neuron* 67(2), 239-252. doi: 10.1016/j.neuron.2010.06.007.

Opazo, P., Sainlos, M., and Choquet, D. (2012). Regulation of AMPA receptor surface diffusion by PSD-95 slots. *Curr Opin Neurobiol* 22(3), 453-460. doi: 10.1016/j.conb.2011.10.010.

Oray, S., Majewska, A., and Sur, M. (2005). Effects of Synaptic Activity on Dendritic Spine Motility of Developing Cortical Layer V Pyramidal Neurons. *Cerebral Cortex* 16(5), 730-741. doi: 10.1093/cercor/bhj019.

Osten, P., Valsamis, L., Harris, A., and Sacktor, T.C. (1996). Protein synthesis-dependent formation of protein kinase Mzeta in long-term potentiation. *J Neurosci* 16(8), 2444-2451.

Osterweil, E., Wells, D.G., and Mooseker, M.S. (2005). A role for myosin VI in postsynaptic structure and glutamate receptor endocytosis. *J Cell Biol* 168(2), 329-338. doi: 10.1083/jcb.200410091.

Ostrowitzki, S., Deptula, D., Thurfjell, L., Barkhof, F., Bohrmann, B., Brooks, D.J., et al. (2012). Mechanism of amyloid removal in patients with Alzheimer disease treated with gantenerumab. *Arch Neurol* 69(2), 198-207. doi: 10.1001/archneurol.2011.1538.

Ota, K.T., Monsey, M.S., Wu, M.S., Young, G.J., and Schafe, G.E. (2010). Synaptic plasticity and NO-cGMP-PKG signaling coordinately regulate ERK-driven gene expression in the lateral amygdala and in the auditory thalamus following Pavlovian fear conditioning. *Learn Mem* 17(4), 221-235. doi: 10.1101/lm.1592510.

Ota, K.T., Pierre, V.J., Ploski, J.E., Queen, K., and Schafe, G.E. (2008). The NO-cGMP-PKG signaling pathway regulates synaptic plasticity and fear memory consolidation in the lateral amygdala via activation of ERK/MAP kinase. *Learning & memory (Cold Spring Harbor, N.Y.)* 15(10), 792-805. doi: 10.1101/lm.1114808.

Otani, S., and Abraham, W.C. (1989). Inhibition of protein synthesis in the dentate gyrus, but not the entorhinal cortex, blocks maintenance of long-term potentiation in rats. *Neuroscience Letters* 106(1), 175-180. doi: https://doi.org/10.1016/0304-3940(89)90222-X.

Otani, S., Bai, J., and Blot, K. (2015). Dopaminergic modulation of synaptic plasticity in rat prefrontal neurons. *Neurosci Bull* 31(2), 183-190. doi: 10.1007/s12264-014-1507-3.

Otani, S., Daniel, H., Roisin, M.P., and Crepel, F. (2003). Dopaminergic modulation of long-term synaptic plasticity in rat prefrontal neurons. *Cereb Cortex* 13(11), 1251-1256. doi: 10.1093/cercor/bhg092.

Otis, T.S., Raman, I.M., and Trussell, L.O. (1995). AMPA receptors with high Ca^{2+} permeability mediate synaptic transmission in the avian auditory pathway. *The Journal of physiology* 482 (Pt 2)(Pt 2), 309-315. doi: 10.1113/jphysiol.1995.sp020519.

Otmakhov, N., Tao-Cheng, J.-H., Carpenter, S., Asrican, B., Dosemeci, A., Reese, T.S., et al. (2004). Persistent Accumulation of Calcium/Calmodulin-Dependent Protein Kinase II in Dendritic Spines after Induction of NMDA Receptor-Dependent Chemical Long-Term Potentiation. *The Journal of Neuroscience* 24(42), 9324-9331. doi: 10.1523/jneurosci.2350-04.2004.

Ouyang, J., Carcea, I., Schiavo, J.K., Jones, K.T., Rabinowitsch, A., Kolaric, R., et al. (2017). Food restriction induces synaptic incorporation of calcium-permeable AMPA receptors in nucleus accumbens. *The European journal of neuroscience* 45(6), 826-836. doi: 10.1111/ejn.13528.

Pachernegg, S., Münster, Y., Muth-Köhne, E., Fuhrmann, G., and Hollmann, M. (2015). GluA2 is rapidly edited at the Q/R site during neural differentiation in vitro. *Frontiers in cellular neuroscience* 9, 69-69. doi: 10.3389/fncel.2015.00069.

Pachernegg, S., Strutz-Seebohm, N., and Hollmann, M. (2012). GluN3 subunit-containing NMDA receptors: not just one-trick ponies. *Trends Neurosci* 35(4), 240-249. doi: 10.1016/j.tins.2011.11.010.

Pak, D.T., Yang, S., Rudolph-Correia, S., Kim, E., and Sheng, M. (2001). Regulation of dendritic spine morphology by SPAR, a PSD-95-associated RapGAP. *Neuron* 31(2), 289-303.

Palmer, T.D., Markakis, E.A., Willhoite, A.R., Safar, F., and Gage, F.H. (1999). Fibroblast Growth Factor-2 Activates a Latent Neurogenic Program in Neural Stem Cells from Diverse Regions of the Adult CNS. *The Journal of Neuroscience* 19(19), 8487-8497. doi: 10.1523/jneurosci.19-19-08487.1999.

Palmeri, A., Ricciarelli, R., Gulisano, W., Rivera, D., Rebosio, C., Calcagno, E., et al. (2017). Amyloid-β Peptide Is Needed for cGMP-Induced Long-Term Potentiation and Memory. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 37(29), 6926-6937. doi: 10.1523/JNEUROSCI.3607-16.2017.

Palmert, M.R., Usiak, M., Mayeux, R., Raskind, M., Tourtellotte, W.W., and Younkin, S.G. (1990). Soluble derivatives of the beta amyloid protein precursor in cerebrospinal fluid: alterations in normal aging and in Alzheimer's disease. *Neurology* 40(7), 1028-1034.

Panchenko, V.A., Glasser, C.R., Partin, K.M., and Mayer, M.L. (1999). Amino acid substitutions in the pore of rat glutamate receptors at sites influencing block by polyamines. *J Physiol* 520 Pt 2, 337-357. doi: 10.1111/j.1469-7793.1999.t01-1-00337.x.

Pandey, K., Yu, X.-W., Steinmetz, A., and Alberini, C.M. (2020). Autophagy coupled to translation is required for long-term memory. *Autophagy*, 1-22. doi: 10.1080/15548627.2020.1775393.

Pandya, N.J., Koopmans, F., Slotman, J.A., Paliukhovich, I., Houtsmuller, A.B., Smit, A.B., et al. (2017). Correlation profiling of brain sub-cellular proteomes reveals co-assembly of synaptic proteins and subcellular distribution. *Scientific Reports* 7(1), 12107. doi: 10.1038/s41598-017-11690-3.

Pandya, N.J., Seeger, C., Babai, N., Gonzalez-Lozano, M.A., Mack, V., Lodder, J.C., et al. (2018). Noelin1 Affects Lateral Mobility of Synaptic AMPA Receptors. *Cell Reports* 24(5), 1218-1230. doi: https://doi.org/10.1016/j.celrep.2018.06.102.

Pang, P.T., Teng, H.K., Zaitsev, E., Woo, N.T., Sakata, K., Zhen, S., et al. (2004). Cleavage of proBDNF by tPA/Plasmin Is Essential for Long-Term Hippocampal Plasticity. *Science* 306(5695), 487-491. doi: 10.1126/science.1100135.

Panja, D., and Bramham, C.R. (2014). BDNF mechanisms in late LTP formation: A synthesis and breakdown. *Neuropharmacology* 76 Pt C, 664-676. doi: 10.1016/j.neuropharm.2013.06.024.

Papa, M., Bundman, M.C., Greenberger, V., and Segal, M. (1995). Morphological analysis of dendritic spine development in primary cultures of hippocampal neurons. *J Neurosci* 15(1 Pt 1), 1-11. doi: 10.1523/jneurosci.15-01-00001.1995.

Pardridge, W.M. (2002). Neurotrophins, neuroprotection and the blood-brain barrier. *Current opinion in investigational drugs (London, England : 2000)* 3(12), 1753-1757.

Pardridge, W.M., Kang, Y.S., and Buciak, J.L. (1994). Transport of human recombinant brain-derived neurotrophic factor (BDNF) through the rat blood-brain barrier in vivo using vector-mediated peptide drug delivery. *Pharm Res* 11(5), 738-746. doi: 10.1023/a:1018940732550.

Parihar, M.S., and Brewer, G.J. (2010). Amyloid Beta as a Modulator of Synaptic Plasticity. *J Alzheimers Dis* 22(3), 741-763. doi: 10.3233/jad-2010-101020.

Park, C.S., Rehrauer, H., and Mansuy, I.M. (2013). Genome-wide analysis of H4K5 acetylation associated with fear memory in mice. *BMC Genomics* 14(1), 539. doi: 10.1186/1471-2164-14-539.

Park, J. (2018a). Phosphorylation of the AMPAR-TARP Complex in Synaptic Plasticity. *Proteomes* 6(4). doi: 10.3390/proteomes6040040.
Park, M. (2018b). AMPA Receptor Trafficking for Postsynaptic Potentiation. *Frontiers in Cellular Neuroscience* 12(361). doi: 10.3389/fncel.2018.00361.
Park, P., Kang, H., Sanderson, T.M., Bortolotto, Z.A., Georgiou, J., Zhuo, M., et al. (2018). The Role of Calcium-Permeable AMPARs in Long-Term Potentiation at Principal Neurons in the Rodent Hippocampus. *Frontiers in synaptic neuroscience* 10, 42-42. doi: 10.3389/fnsyn.2018.00042.
Park, P., Kang, H., Sanderson, T.M., Bortolotto, Z.A., Georgiou, J., Zhuo, M., et al. (2019a). On the Role of Calcium-Permeable AMPARs in Long-Term Potentiation and Synaptic Tagging in the Rodent Hippocampus. *Frontiers in synaptic neuroscience* 11, 4-4. doi: 10.3389/fnsyn.2019.00004.
Park, P., Ko, K.-H., Zhuo, M., Kaang, B.-K., and Collingridge, G. (2019b). Calcium-permeable AMPA receptors mediate the increased unitary conductance during LTP in the hippocampus. *IBRO Reports* 6, S533. doi: https://doi.org/10.1016/j.ibror.2019.07.1661.
Park, P., Sanderson, T.M., Amici, M., Choi, S.-L., Bortolotto, Z.A., Zhuo, M., et al. (2016). Calcium-Permeable AMPA Receptors Mediate the Induction of the Protein Kinase A-Dependent Component of Long-Term Potentiation in the Hippocampus. *The Journal of Neuroscience* 36(2), 622-631. doi: 10.1523/jneurosci.3625-15.2016.
Park, S., Park, J.M., Kim, S., Kim, J.-A., Shepherd, J.D., Smith-Hicks, C.L., et al. (2008). Elongation Factor 2 and Fragile X Mental Retardation Protein Control the Dynamic Translation of Arc/Arg3.1 Essential for mGluR-LTD. *Neuron* 59(1), 70-83. doi: 10.1016/j.neuron.2008.05.023.
Parnavelas, J.G., Globus, A., and Kaups, P. (1973). Continuous illumination from birth affects spine density of neurons in the visual cortex of the rat. *Experimental Neurology* 40(3), 742-747. doi: https://doi.org/10.1016/0014-4886(73)90108-8.
Passafaro, M., Piech, V., and Sheng, M. (2001). Subunit-specific temporal and spatial patterns of AMPA receptor exocytosis in hippocampal neurons. *Nat Neurosci* 4(9), 917-926. doi: 10.1038/nn0901-917.
Pastalkova, E., Serrano, P., Pinkhasova, D., Wallace, E., Fenton, A.A., and Sacktor, T.C. (2006). Storage of Spatial Information by the Maintenance Mechanism of LTP. *Science* 313(5790), 1141-1144. doi: 10.1126/science.1128657.
Pastuzyn, E.D., Day, C.E., Kearns, R.B., Kyrke-Smith, M., Taibi, A.V., McCormick, J., et al. (2018). The Neuronal Gene Arc Encodes a Repurposed Retrotransposon Gag Protein that Mediates Intercellular RNA Transfer. *Cell* 172(1-2), 275-288.e218. doi: 10.1016/j.cell.2017.12.024.
Patneau, D.K., Wright, P.W., Winters, C., Mayer, M.L., and Gallo, V. (1994). Glial cells of the oligodendrocyte lineage express both kainate- and AMPA-preferring subtypes of glutamate receptor. *Neuron* 12(2), 357-371.
Patterson, S.L., Abel, T., Deuel, T.A.S., Martin, K.C., Rose, J.C., and Kandel, E.R. (1996). Recombinant BDNF Rescues Deficits in Basal Synaptic Transmission and Hippocampal LTP in BDNF Knockout Mice. *Neuron* 16(6), 1137-1145. doi: https://doi.org/10.1016/S0896-6273(00)80140-3.
Paul, C., Schöberl, F., Weinmeister, P., Micale, V., Wotjak, C.T., Hofmann, F., et al. (2008). Signaling through cGMP-dependent protein kinase I in the amygdala is critical for auditory-cued fear memory and long-term potentiation. *J Neurosci* 28(52), 14202-14212. doi: 10.1523/jneurosci.2216-08.2008.
Pavlopoulos, E., Trifilieff, P., Chevaleyre, V., Fioriti, L., Zairis, S., Pagano, A., et al. (2011). Neuralized1 Activates CPEB3: A Function for Nonproteolytic Ubiquitin in Synaptic Plasticity and Memory Storage. *Cell* 147(6), 1369-1383. doi: https://doi.org/10.1016/j.cell.2011.09.056.
Pavlov, I.P., and Gantt, W. (1928). Lectures on conditioned reflexes: Twenty-five years of objective study of the higher nervous activity (behaviour) of animals.
Pawlik, M., Sastre, M., Calero, M., Mathews, P.M., Schmidt, S.D., Nixon, R.A., et al. (2004). Overexpression of human cystatin C in transgenic mice does not affect levels of endogenous brain amyloid Beta Peptide. *J Mol Neurosci* 22(1-2), 13-18. doi: 10.1385/jmn:22:1-2:13.

Peebles, C.L., Yoo, J., Thwin, M.T., Palop, J.J., Noebels, J.L., and Finkbeiner, S. (2010). Arc regulates spine morphology and maintains network stability in vivo. *Proceedings of the National Academy of Sciences* 107(42), 18173-18178. doi: 10.1073/pnas.1006546107.

Pelkey, Kenneth A., Barksdale, E., Craig, Michael T., Yuan, X., Sukumaran, M., Vargish, Geoffrey A., et al. (2015). Pentraxins Coordinate Excitatory Synapse Maturation and Circuit Integration of Parvalbumin Interneurons. *Neuron* 85(6), 1257-1272. doi: https://doi.org/10.1016/j.neuron.2015.02.020.

Pen, Y., Borovok, N., Reichenstein, M., Sheinin, A., and Michaelevski, I. (2016). Membrane-tethered AKT kinase regulates basal synaptic transmission and early phase LTP expression by modulation of post-synaptic AMPA receptor level. *Hippocampus* 26(9), 1149-1167. doi: 10.1002/hipo.22597.

Penfield, W., And Boldrey, E. (1937). Somatic Motor And Sensory Representation In The Cerebral Cortex Of Man As Studied By Electrical Stimulation1. *Brain* 60(4), 389-443. Doi: 10.1093/Brain/60.4.389.

Penfield, W., and Milner, B. (1958). Memory deficit produced by bilateral lesions in the hippocampal zone. *AMA Arch Neurol Psychiatry* 79(5), 475-497.

Penke, Z., Chagneau, C., and Laroche, S. (2011). Contribution of Egr1/zif268 to Activity-Dependent Arc/Arg3.1 Transcription in the Dentate Gyrus and Area CA1 of the Hippocampus. *Frontiers in Behavioral Neuroscience* 5(48). doi: 10.3389/fnbeh.2011.00048.

Penn, A.C., Williams, S.R., and Greger, I.H. (2008). Gating motions underlie AMPA receptor secretion from the endoplasmic reticulum. *The EMBO Journal* 27(22), 3056-3068. doi: 10.1038/emboj.2008.222.

Penn, A.C., Zhang, C.L., Georges, F., Royer, L., Breillat, C., Hosy, E., et al. (2017). Hippocampal LTP and contextual learning require surface diffusion of AMPA receptors. *Nature*. doi: 10.1038/nature23658.

Pérez-Otaño, I., Larsen, R.S., and Wesseling, J.F. (2016). Emerging roles of GluN3-containing NMDA receptors in the CNS. *Nature Reviews Neuroscience* 17, 623. doi: 10.1038/nrn.2016.92.

Perneczky, R., Guo, L.H., Kagerbauer, S.M., Werle, L., Kurz, A., Martin, J., et al. (2013). Soluble amyloid precursor protein β as blood-based biomarker of Alzheimer's disease. *Transl Psychiatry* 3(2), e227. doi: 10.1038/tp.2013.11.

Perneczky, R., Tsolakidou, A., Arnold, A., Diehl-Schmid, J., Grimmer, T., Förstl, H., et al. (2011). CSF soluble amyloid precursor proteins in the diagnosis of incipient Alzheimer disease. *Neurology* 77(1), 35-38. doi: 10.1212/WNL.0b013e318221ad47.

Peters-Libeu, C., Campagna, J., Mitsumori, M., Poksay, K.S., Spilman, P., Sabogal, A., et al. (2015). sAβPPα is a Potent Endogenous Inhibitor of BACE1. *J Alzheimers Dis* 47(3), 545-555. doi: 10.3233/jad-150282.

Petersen, J.D., Chen, X., Vinade, L., Dosemeci, A., Lisman, J.E., and Reese, T.S. (2003). Distribution of postsynaptic density (PSD)-95 and Ca^{2+}/calmodulin-dependent protein kinase II at the PSD. *J Neurosci* 23(35), 11270-11278.

Petralia, R.S., Esteban, J.A., Wang, Y.X., Partridge, J.G., Zhao, H.M., Wenthold, R.J., et al. (1999). Selective acquisition of AMPA receptors over postnatal development suggests a molecular basis for silent synapses. *Nat Neurosci* 2(1), 31-36. doi: 10.1038/4532.

Petrini, E.M., Lu, J., Cognet, L., Lounis, B., Ehlers, M.D., and Choquet, D. (2009). Endocytic Trafficking and Recycling Maintain a Pool of Mobile Surface AMPA Receptors Required for Synaptic Potentiation. *Neuron* 63(1), 92-105. doi: 10.1016/j.neuron.2009.05.025.

Pettit, D.L., Shao, Z., and Yakel, J.L. (2001). beta-Amyloid(1-42) peptide directly modulates nicotinic receptors in the rat hippocampal slice. *J Neurosci* 21(1), Rc120. doi: 10.1523/JNEUROSCI.21-01-j0003.2001.

Phinney, A.L., Drisaldi, B., Schmidt, S.D., Lugowski, S., Coronado, V., Liang, Y., et al. (2003). In vivo reduction of amyloid-beta by a mutant copper transporter. *Proc Natl Acad Sci U S A* 100(24), 14193-14198. doi: 10.1073/pnas.2332851100.

Piaceri, I., Nacmias, B., and Sorbi, S. (2013). Genetics of familial and sporadic Alzheimer's disease. *Front Biosci (Elite Ed)* 5, 167-177. doi: 10.2741/e605.

Picconi, B., Bagetta, V., Ghiglieri, V., Paillè, V., Di Filippo, M., Pendolino, V., et al. (2010). Inhibition of phosphodiesterases rescues striatal long-term depression and reduces levodopa-induced dyskinesia. *Brain* 134(2), 375-387. doi: 10.1093/brain/awq342.

Picconi, B., Bagetta, V., Ghiglieri, V., Paillè, V., Di Filippo, M., Pendolino, V., et al. (2011). Inhibition of phosphodiesterases rescues striatal long-term depression and reduces levodopa-induced dyskinesia. *Brain* 134(Pt 2), 375-387. doi: 10.1093/brain/awq342.

Pick, J.E., Khatri, L., Sathler, M.F., and Ziff, E.B. (2017). mGluR long-term depression regulates GluA2 association with COPII vesicles and exit from the endoplasmic reticulum. *The EMBO journal* 36(2), 232-244.

Pickard, L., Noël, J., Henley, J.M., Collingridge, G.L., and Molnar, E. (2000). Developmental Changes in Synaptic AMPA and NMDA Receptor Distribution and AMPA Receptor Subunit Composition in Living Hippocampal Neurons. *The Journal of Neuroscience* 20(21), 7922-7931. doi: 10.1523/jneurosci.20-21-07922.2000.

Pidoplichko, V.I., Prager, E.M., Aroniadou-Anderjaska, V., and Braga, M.F.M. (2013). α7-Containing nicotinic acetylcholine receptors on interneurons of the basolateral amygdala and their role in the regulation of the network excitability. *Journal of neurophysiology* 110(10), 2358-2369. doi: 10.1152/jn.01030.2012.

Pierce, J.E., Trojanowski, J.Q., Graham, D.I., Smith, D.H., and McIntosh, T.K. (1996). Immunohistochemical characterization of alterations in the distribution of amyloid precursor proteins and beta-amyloid peptide after experimental brain injury in the rat. *J Neurosci* 16(3), 1083-1090.

Pierce, V.D., and Niu, L. (2019). Stargazin and γ4 slow the channel opening and closing rates of GluA4 AMPA receptors. *Scientific Reports* 9(1), 9570. doi: 10.1038/s41598-019-45870-0.

Pike, C.J., Cummings, B.J., and Cotman, C.W. (1992). beta-Amyloid induces neuritic dystrophy in vitro: similarities with Alzheimer pathology. *Neuroreport* 3(9), 769-772. doi: 10.1097/00001756-199209000-00012.

Plant, K., Pelkey, K.A., Bortolotto, Z.A., Morita, D., Terashima, A., McBain, C.J., et al. (2006). Transient incorporation of native GluR2-lacking AMPA receptors during hippocampal long-term potentiation. *Nat Neurosci* 9(5), 602-604. doi: 10.1038/nn1678.

Plath, N., Ohana, O., Dammermann, B., Errington, M.L., Schmitz, D., Gross, C., et al. (2006). Arc/Arg3.1 is essential for the consolidation of synaptic plasticity and memories. *Neuron* 52(3), 437-444. doi: 10.1016/j.neuron.2006.08.024.

Plummer, S., Van den Heuvel, C., Thornton, E., Corrigan, F., and Cappai, R. (2016). The Neuroprotective Properties of the Amyloid Precursor Protein Following Traumatic Brain Injury. *Aging Dis* 7(2), 163-179. doi: 10.14336/ad.2015.0907.

Polleux, F., and Snider, W. (2010). Initiating and Growing an Axon. *Cold Spring Harbor Perspectives in Biology* 2(4). doi: 10.1101/cshperspect.a001925.

Poncer, J.C., Esteban, J.A., and Malinow, R. (2002). Multiple Mechanisms for the Potentiation of AMPA Receptor-Mediated Transmission by α-Ca²⁺/Calmodulin-Dependent Protein Kinase II. *The Journal of Neuroscience* 22(11), 4406-4411. doi: 10.1523/jneurosci.22-11-04406.2002.

Poncer, J.C., Esteban, J.A., and Malinow, R. (2002). Multiple Mechanisms for the Potentiation of AMPA Receptor-Mediated Transmission by α-Ca^{2+}/Calmodulin-Dependent Protein Kinase II. *The Journal of Neuroscience* 22(11), 4406-4411. doi: 10.1523/jneurosci.22-11-04406.2002.

Popov, V.I., Davies, H.A., Rogachevsky, V.V., Patrushev, I.V., Errington, M.L., Gabbott, P.L.A., et al. (2004). Remodelling of synaptic morphology but unchanged synaptic density during late phase long-term potentiation(ltp): A serial section electron micrograph study in the dentate gyrus in the anaesthetised rat. *Neuroscience* 128(2), 251-262. doi: https://doi.org/10.1016/j.neuroscience.2004.06.029.

Portelius, E., Andreasson, U., Ringman, J.M., Buerger, K., Daborg, J., Buchhave, P., et al. (2010). Distinct cerebrospinal fluid amyloid beta peptide signatures in sporadic and PSEN1 A431E-associated familial Alzheimer's disease. *Molecular neurodegeneration* 5, 2-2. doi: 10.1186/1750-1326-5-2.

Portelius, E., Fortea, J., Molinuevo, J.L., Gustavsson, M.K., Andreasson, U., and Sanchez-Valle, R. (2012). The amyloid-β isoform pattern in cerebrospinal fluid in familial PSEN1 M139T- and L286P-associated Alzheimer's disease. *Mol Med Rep* 5(4), 1111-1115. doi: 10.3892/mmr.2012.774.

Porter, J.T., and McCarthy, K.D. (1995). GFAP-positive hippocampal astrocytes in situ respond to glutamatergic neuroligands with increases in $[Ca^{2+}]i$. *Glia* 13(2), 101-112. doi: 10.1002/glia.440130204.

Pozo, K., and Goda, Y. (2010). Unraveling mechanisms of homeostatic synaptic plasticity. *Neuron* 66(3), 337-351. doi: 10.1016/j.neuron.2010.04.028.

Prior, R., Monning, U., Schreiter-Gasser, U., Weidemann, A., Blennow, K., Gottfries, C.G., et al. (1991). Quantitative changes in the amyloid beta A4 precursor protein in Alzheimer cerebrospinal fluid. *Neurosci Lett* 124(1), 69-73.

Pulford, B., Reim, N., Bell, A., Veatch, J., Forster, G., Bender, H., et al. (2010). Liposome-siRNA-peptide complexes cross the blood-brain barrier and significantly decrease PrP on neuronal cells and PrP in infected cell cultures. *PloS one* 5(6), e11085-e11085. doi: 10.1371/journal.pone.0011085.

Purkey, A.M., and Dell'Acqua, M.L. (2020). Phosphorylation-Dependent Regulation of Ca^{2+}-Permeable AMPA Receptors During Hippocampal Synaptic Plasticity. *Frontiers in Synaptic Neuroscience* 12(8). doi: 10.3389/fnsyn.2020.00008.

Puzzo, D., Privitera, L., Leznik, E., Fa, M., Staniszewski, A., Palmeri, A., et al. (2008). Picomolar amyloid-beta positively modulates synaptic plasticity and memory in hippocampus. *J Neurosci* 28(53), 14537-14545. doi: 10.1523/jneurosci.2692-08.2008.

Puzzo, D., Staniszewski, A., Deng, S.X., Privitera, L., Leznik, E., Liu, S., et al. (2009). Phosphodiesterase 5 Inhibition Improves Synaptic Function, Memory, and Amyloid-β Load in an Alzheimer's Disease Mouse Model. *The Journal of Neuroscience* 29(25), 8075-8086. doi: 10.1523/jneurosci.0864-09.2009.

Qi, M., Zhuo, M., Skålhegg, B.S., Brandon, E.P., Kandel, E.R., McKnight, G.S., et al. (1996). Impaired hippocampal plasticity in mice lacking the Cbeta1 catalytic subunit of cAMP-dependent protein kinase. *Proceedings of the National Academy of Sciences* 93(4), 1571-1576.

Rabinowitch, I., and Segev, I. (2006). The interplay between homeostatic synaptic plasticity and functional dendritic compartments. *J Neurophysiol* 96(1), 276-283. doi: 10.1152/jn.00074.2006.

Rabinowitch, I., and Segev, I. (2008). Two opposing plasticity mechanisms pulling a single synapse. *Trends Neurosci* 31(8), 377-383. doi: 10.1016/j.tins.2008.05.005.

Radcliffe, K.A., Fisher, J.L., Gray, R., And Dani, J.A. (1999). Nicotinic Modulation of Glutamate and GABA Synaptic Transmission in Hippocampal Neurons. *Annals of the New York Academy of Sciences* 868(1), 591-610. doi: 10.1111/j.1749-6632.1999.tb11332.x.

Radley, J.J., Farb, C.R., He, Y., Janssen, W.G.M., Rodrigues, S.M., Johnson, L.R., et al. (2007). Distribution of NMDA and AMPA receptor subunits at thalamo-amygdaloid dendritic spines. *Brain research* 1134(1), 87-94. doi: 10.1016/j.brainres.2006.11.045.

Rameau, G.A., Tukey, D.S., Garcin-Hosfield, E.D., Titcombe, R.F., Misra, C., Khatri, L., et al. (2007). Biphasic coupling of neuronal nitric oxide synthase phosphorylation to the NMDA receptor regulates AMPA receptor trafficking and neuronal cell death. *J Neurosci* 27(13), 3445-3455. doi: 10.1523/jneurosci.4799-06.2007.

Ramirez-Amaya, V., Angulo-Perkins, A., Chawla, M.K., Barnes, C.A., and Rosi, S. (2013). Sustained Transcription of the Immediate Early Gene Arc in the Dentate Gyrus after Spatial Exploration. *The Journal of Neuroscience* 33(4), 1631-1639. doi: 10.1523/jneurosci.2916-12.2013.

Ramiro-Cortés, Y., and Israely, I. (2013). Long Lasting Protein Synthesis- and Activity-Dependent Spine Shrinkage and Elimination after Synaptic Depression. *PLOS ONE* 8(8), e71155. doi: 10.1371/journal.pone.0071155.

Ramón y Cajal, S. (1888). Estructura del cerebelo. *Gaceta Medica Catalana XI (15)* 15.

Ramón y Cajal, S. (1909). *Histologie du système nerveux de l'homme & des vertébrés.* Paris :: Maloine.

Rana, S., Yue, S., Stadel, D., and Zöller, M. (2012). Toward tailored exosomes: The exosomal tetraspanin web contributes to target cell selection. *The International Journal of Biochemistry & Cell Biology* 44(9), 1574-1584. doi: https://doi.org/10.1016/j.biocel.2012.06.018.

Ranschburg, P. (1911). *Das kranke Gedächtnis: Ergebnisse und Methoden der experimentellen Erforschung der alltäglichen Falschleistungen und der Pathologie des Gedächtnisses.* Barth.

Rao, A., and Craig, A.M. (1997). Activity Regulates the Synaptic Localization of the NMDA Receptor in Hippocampal Neurons. *Neuron* 19(4), 801-812. doi: https://doi.org/10.1016/S0896-6273(00)80962-9.

Rasmussen, P., Brassard, P., Adser, H., Pedersen, M.V., Leick, L., Hart, E., et al. (2009). Evidence for a release of brain-derived neurotrophic factor from the brain during exercise. *Experimental Physiology* 94(10), 1062-1069. doi: 10.1113/expphysiol.2009.048512.

Rauner, C., and Köhr, G. (2011). Triheteromeric NR1/NR2A/NR2B receptors constitute the major N-methyl-D-aspartate receptor population in adult hippocampal synapses. *J Biol Chem* 286(9), 7558-7566. doi: 10.1074/jbc.M110.182600.

Ray, B., Maloney, B., Sambamurti, K., Karnati, H.k., Nelson, P.T., Greig, N.H., et al. (2020). Rivastigmine modifies the α-secretase pathway and potentially early Alzheimer's disease. *Translational Psychiatry* 10(1), 47. doi: 10.1038/s41398-020-0709-x.

Ray, J., and Gage, F.H. (2006). Differential properties of adult rat and mouse brain-derived neural stem/progenitor cells. *Molecular and Cellular Neuroscience* 31(3), 560-573. doi: https://doi.org/10.1016/j.mcn.2005.11.010.

Raymond, C.R., Thompson, V.L., Tate, W.P., and Abraham, W.C. (2000). Metabotropic Glutamate Receptors Trigger Homosynaptic Protein Synthesis to Prolong Long-Term Potentiation. *The Journal of Neuroscience* 20(3), 969-976. doi: 10.1523/jneurosci.20-03-00969.2000.

Raymond, C.R., Thompson, V.L., Tate, W.P., and Abraham, W.C. (2000). Metabotropic Glutamate Receptors Trigger Homosynaptic Protein Synthesis to Prolong Long-Term Potentiation. *The Journal of Neuroscience* 20(3), 969-976. doi: 10.1523/jneurosci.20-03-00969.2000.

Rebola, N., Carta, M., Lanore, F., Blanchet, C., and Mulle, C. (2011). NMDA receptor–dependent metaplasticity at hippocampal mossy fiber synapses. *Nature Neuroscience* 14(6), 691-693. doi: 10.1038/nn.2809.

Regehr, W.G. (2012). Short-Term Presynaptic Plasticity. *Cold Spring Harbor Perspectives in Biology* 4(7), a005702. doi: 10.1101/cshperspect.a005702.

Reger, A.S., Yang, M.P., Koide-Yoshida, S., Guo, E., Mehta, S., Yuasa, K., et al. (2014). Crystal structure of the cGMP-dependent protein kinase II leucine zipper and Rab11b protein complex reveals molecular details of G-kinase-specific interactions. *J Biol Chem* 289(37), 25393-25403. doi: 10.1074/jbc.M114.575894.

Reierson, G.W., Guo, S., Mastronardi, C., Licinio, J., and Wong, M.L. (2011). cGMP Signaling, Phosphodiesterases and Major Depressive Disorder. *Curr Neuropharmacol* 9(4), 715-727. doi: 10.2174/157015911798376271.

Reilly, J.E., Hanson, H.H., Fernández-Monreal, M., Wearne, S.L., Hof, P.R., and Phillips, G.R. (2011). Characterization of MSB Synapses in Dissociated Hippocampal Culture with Simultaneous Pre- and Postsynaptic Live Microscopy. *PLOS ONE* 6(10), e26478. doi: 10.1371/journal.pone.0026478.

Reinders, N.R., Pao, Y., Renner, M.C., da Silva-Matos, C.M., Lodder, T.R., Malinow, R., et al. (2016). Amyloid-beta effects on synapses and memory require AMPA receptor subunit GluA3. *Proc Natl Acad Sci U S A* 113(42), E6526-e6534. doi: 10.1073/pnas.1614249113.

Reisel, D., Bannerman, D.M., Schmitt, W.B., Deacon, R.M., Flint, J., Borchardt, T., et al. (2002). Spatial memory dissociations in mice lacking GluR1. *Nat Neurosci* 5(9), 868-873. doi: 10.1038/nn910.

Ren, S.-Q., Yan, J.-Z., Zhang, X.-Y., Bu, Y.-F., Pan, W.-W., Yao, W., et al. (2013). PKCλ is critical in AMPA receptor phosphorylation and synaptic incorporation during LTP. *The EMBO Journal* 32(10), 1365-1380. doi: 10.1038/emboj.2013.60.

Renner, M.C., Albers, E.H.H., Gutierrez-Castellanos, N., Reinders, N.R., van Huijstee, A.N., Xiong, H., et al. (2017). Synaptic plasticity through activation of GluA3-containing AMPA-receptors. *eLife* 6. doi: 10.7554/eLife.25462.

Reymann, K.G., Schulzeck, K., Kase, H., and Matthies, H. (1988). Phorbol ester-induced hippocampal long-term potentiation is counteracted by inhibitors of protein kinase C. *Exp Brain Res* 71(1), 227-230.

Rial Verde, E.M., Lee-Osbourne, J., Worley, P.F., Malinow, R., and Cline, H.T. (2006). Increased Expression of the Immediate-Early Gene Arc/Arg3.1 Reduces AMPA Receptor-Mediated Synaptic Transmission. *Neuron* 52(3), 461-474. doi: 10.1016/j.neuron.2006.09.031.

Ribot, T. (1882). *Diseases of memory: An essay in the positive psychology.* D. Appleton.

Ricciarelli, R., and Fedele, E. (2017). The Amyloid Cascade Hypothesis in Alzheimer's Disease: It's Time to Change Our Mind. *Current neuropharmacology* 15(6), 926-935. doi: 10.2174/1570159X15666170116143743.

Rice, H.C., de Malmazet, D., Schreurs, A., Frere, S., Van Molle, I., Volkov, A.N., et al. (2019). Secreted amyloid-β precursor protein functions as a $GABA_BR1a$ ligand to modulate synaptic transmission. *Science* 363(6423), eaao4827. doi: 10.1126/science.aao4827.

Rice, H.C., Wierda, K.D., Frere, S., Vertkin, I., Van Molle, I., Ranaivoson, F., Et Al. (2017). Soluble Amyloid Precursor Protein Is An Isoform-Specific Gaba(B) Receptor Ligand That Suppresses Synaptic Release Probability. *Alzheimer's & Dementia: The Journal Of The Alzheimer's Association* 13(7), P206. Doi: 10.1016/J.Jalz.2017.07.075.

Richmond, S.A., Irving, A.J., Molnar, E., McIlhinney, R.A., Michelangeli, F., Henley, J.M., et al. (1996). Localization of the glutamate receptor subunit GluR1 on the surface of living and within cultured hippocampal neurons. *Neuroscience* 75(1), 69-82.

Richter, M.C., Ludewig, S., Winschel, A., Abel, T., Bold, C., Salzburger, L.R., et al. (2018a). Distinct in vivo roles of secreted APP ectodomain variants APPsα and APPsβ in regulation of spine density, synaptic plasticity, and cognition. *The EMBO Journal* 37(11), e98335. doi: 10.15252/embj.201798335.

Ring, S., Weyer, S.W., Kilian, S.B., Waldron, E., Pietrzik, C.U., Filippov, M.A., et al. (2007a). The secreted beta-amyloid precursor protein ectodomain APPs alpha is sufficient to rescue the anatomical, behavioral, and electrophysiological abnormalities of APP-deficient mice. *J Neurosci* 27(29), 7817-7826. doi: 10.1523/jneurosci.1026-07.2007.

Rinne, J.O., Brooks, D.J., Rossor, M.N., Fox, N.C., Bullock, R., Klunk, W.E., et al. (2010). 11C-PiB PET assessment of change in fibrillar amyloid-beta load in patients with Alzheimer's disease treated with bapineuzumab: a phase 2, double-blind, placebo-controlled, ascending-dose study. *Lancet Neurol* 9(4), 363-372. doi: 10.1016/s1474-4422(10)70043-0.

Ritter, L.M., Vazquez, D.M., and Meador-Woodruff, J.H. (2002). Ontogeny of ionotropic glutamate receptor subunit expression in the rat hippocampus. *Brain Res Dev Brain Res* 139(2), 227-236. doi: 10.1016/s0165-3806(02)00572-2.

Rivera-Olvera, A., Rodríguez-Durán, L.F., and Escobar, M.L. (2016). Conditioned taste aversion prevents the long-lasting BDNF-induced enhancement of synaptic transmission in the insular cortex: A metaplastic effect. *Neurobiol Learn Mem* 130, 71-76. doi: 10.1016/j.nlm.2016.01.014.

Rizzoli, S.O., and Betz, W.J. (2005). Synaptic vesicle pools. *Nat Rev Neurosci* 6(1), 57-69. doi: 10.1038/nrn1583.

Roberts, L.A., Large, C.H., O'Shaughnessy, C.T., and Morris, B.J. (1997). Long-term potentiation in perforant path/granule cell synapses is associated with a post-synaptic induction of

proenkephalin gene expression. *Neurosci Lett* 227(3), 205-208. doi: 10.1016/s0304-3940(97)00349-2.

Robertson, G., Bushell, T.J., and Zagnoni, M. (2014). Chemically induced synaptic activity between mixed primary hippocampal co-cultures in a microfluidic system. *Integr Biol (Camb)* 6(6), 636-644. doi: 10.1039/c3ib40221e.

Roche, K.W., O'Brien, R.J., Mammen, A.L., Bernhardt, J., and Huganir, R.L. (1996). Characterization of multiple phosphorylation sites on the AMPA receptor GluR1 subunit. *Neuron* 16(6), 1179-1188. doi: 10.1016/s0896-6273(00)80144-0.

Rolstad, S., Jakobsson, J., Sellgren, C., Ekman, C.-J., Blennow, K., Zetterberg, H., et al. (2015). Cognitive Performance and Cerebrospinal Fluid Biomarkers of Neurodegeneration: A Study of Patients with Bipolar Disorder and Healthy Controls. *PLOS ONE* 10(5), e0127100. doi: 10.1371/journal.pone.0127100.

Rongo, C. (2002). A fresh look at the role of CaMKII in hippocampal synaptic plasticity and memory. *BioEssays* 24(3), 223-233. doi: 10.1002/bies.10057.

Rosen, C., Andreasson, U., Mattsson, N., Marcusson, J., Minthon, L., Andreasen, N., et al. (2012). Cerebrospinal fluid profiles of amyloid beta-related biomarkers in Alzheimer's disease. *Neuromolecular Med* 14(1), 65-73. doi: 10.1007/s12017-012-8171-4.

Rosén, C., Andreasson, U., Mattsson, N., Marcusson, J., Minthon, L., Andreasen, N., et al. (2012). Cerebrospinal fluid profiles of amyloid β-related biomarkers in Alzheimer's disease. *Neuromolecular Med* 14(1), 65-73. doi: 10.1007/s12017-012-8171-4.

Rosi, S., Ramirez-Amaya, V., Vazdarjanova, A., Worley, P.F., Barnes, C.A., and Wenk, G.L. (2005). Neuroinflammation alters the hippocampal pattern of behaviorally induced Arc expression. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 25(3), 723-731. doi: 10.1523/JNEUROSCI.4469-04.2005.

Ross, F.M., Allan, S.M., Rothwell, N.J., and Verkhratsky, A. (2003). A dual role for interleukin-1 in LTP in mouse hippocampal slices. *J Neuroimmunol* 144(1-2), 61-67. doi: 10.1016/j.jneuroim.2003.08.030.

Ross, S.P., Baker, K.E., Fisher, A., Hoff, L., Pak, E.S., and Murashov, A.K. (2018). miRNA-431 Prevents Amyloid-β-Induced Synapse Loss in Neuronal Cell Culture Model of Alzheimer's Disease by Silencing Kremen1. *Frontiers in Cellular Neuroscience* 12(87). doi: 10.3389/fncel.2018.00087.

Rossjohn, J., Cappai, R., Feil, S.C., Henry, A., McKinstry, W.J., Galatis, D., et al. (1999). Crystal structure of the N-terminal, growth factor-like domain of Alzheimer amyloid precursor protein. *Nat Struct Biol* 6(4), 327-331. doi: 10.1038/7562.

Routh, B.N., Johnston, D., Harris, K., and Chitwood, R.A. (2009). Anatomical and Electrophysiological Comparison of CA1 Pyramidal Neurons of the Rat and Mouse. *Journal of Neurophysiology* 102(4), 2288-2302. doi: 10.1152/jn.00082.2009.

Royer, S., Sirota, A., Patel, J., and Buzsáki, G. (2010). Distinct representations and theta dynamics in dorsal and ventral hippocampus. *J Neurosci* 30(5), 1777-1787. doi: 10.1523/jneurosci.4681-09.2010.

Rozmahel, R., Huang, J., Chen, F., Liang, Y., Nguyen, V., Ikeda, M., et al. (2002). Normal brain development in PS1 hypomorphic mice with markedly reduced gamma-secretase cleavage of betaAPP. *Neurobiol Aging* 23(2), 187-194. doi: 10.1016/s0197-4580(01)00267-6.

Rozov, A., Sprengel, R., and Seeburg, P.H. (2012). GluA2-lacking AMPA receptors in hippocampal CA1 cell synapses: evidence from gene-targeted mice. *Front Mol Neurosci* 5, 22. doi: 10.3389/fnmol.2012.00022.

Rozov, A., Zilberter, Y., Wollmuth, L.P., and Burnashev, N. (1998). Facilitation of currents through rat Ca^{2+}-permeable AMPA receptor channels by activity-dependent relief from polyamine block. *J Physiol* 511 (Pt 2)(Pt 2), 361-377. doi: 10.1111/j.1469-7793.1998.361bh.x.

Rubio, M.E., and Wenthold, R.J. (1999). Calnexin and the immunoglobulin binding protein (BiP) coimmunoprecipitate with AMPA receptors. *J Neurochem* 73(3), 942-948. doi: 10.1046/j.1471-4159.1999.0730942.x.

Rumpel, S., LeDoux, J., Zador, A., and Malinow, R. (2005). Postsynaptic Receptor Trafficking Underlying a Form of Associative Learning. *Science* 308(5718), 83-88. doi: 10.1126/science.1103944.

Rust, M.B., Gurniak, C.B., Renner, M., Vara, H., Morando, L., Görlich, A., et al. (2010). Learning, AMPA receptor mobility and synaptic plasticity depend on n-cofilin-mediated actin dynamics. *Embo j* 29(11), 1889-1902. doi: 10.1038/emboj.2010.72.

Ryan, M.M., Mason-Parker, S.E., Tate, W.P., Abraham, W.C., and Williams, J.M. (2011). Rapidly induced gene networks following induction of long-term potentiation at perforant path synapses in vivo. *Hippocampus* 21(5), 541-553. doi: 10.1002/hipo.20770.

Ryan, M.M., Morris, G.P., Mockett, B.G., Bourne, K., Abraham, W.C., Tate, W.P., et al. (2013). Time-dependent changes in gene expression induced by secreted amyloid precursor protein-alpha in the rat hippocampus. *BMC Genomics* 14, 376. doi: 10.1186/1471-2164-14-376.

Saavedra, A., Giralt, A., Arumí, H., Alberch, J., and Pérez-Navarro, E. (2013). Regulation of Hippocampal cGMP Levels as a Candidate to Treat Cognitive Deficits in Huntington's Disease. *PLOS ONE* 8(9), e73664. doi: 10.1371/journal.pone.0073664.

Sabatini, B.L., Oertner, T.G., and Svoboda, K. (2002). The life cycle of Ca(2+) ions in dendritic spines. *Neuron* 33(3), 439-452.

Sabbagh, M.N. (2009). Drug development for Alzheimer's disease: where are we now and where are we headed? *The American journal of geriatric pharmacotherapy* 7(3), 167-185. doi: 10.1016/j.amjopharm.2009.06.003.

Sagata, N., Iwaki, A., Aramaki, T., Takao, K., Kura, S., Tsuzuki, T., et al. (2010). Comprehensive behavioural study of GluR4 knockout mice: implication in cognitive function. *Genes, Brain and Behavior* 9(8), 899-909. doi: 10.1111/j.1601-183X.2010.00629.x.

Sahu, Madhusmita P., Nikkilä, O., Lågas, S., Kolehmainen, S., and Castrén, E. (2019). Culturing primary neurons from rat hippocampus and cortex. *Neuronal Signaling* 3(2). doi: 10.1042/ns20180207.

Saint-Pol, J., Gosselet, F., Duban-Deweer, S., Pottiez, G., and Karamanos, Y. (2020). Targeting and Crossing the Blood-Brain Barrier with Extracellular Vesicles. *Cells* 9(4). doi: 10.3390/cells9040851.

Sajdyk, T.J., Johnson, P.L., Leitermann, R.J., Fitz, S.D., Dietrich, A., Morin, M., et al. (2008). Neuropeptide Y in the Amygdala Induces Long-Term Resilience to Stress-Induced Reductions in Social Responses But Not Hypothalamic–Adrenal–Pituitary Axis Activity or Hyperthermia. *The Journal of Neuroscience* 28(4), 893-903. doi: 10.1523/jneurosci.0659-07.2008.

Sajikumar, S., and Frey, J.U. (2003). Anisomycin inhibits the late maintenance of long-term depression in rat hippocampal slices in vitro. *Neurosci Lett* 338(2), 147-150. doi: 10.1016/s0304-3940(02)01400-3.

Sajikumar, S., and Frey, J.U. (2004). Late-associativity, synaptic tagging, and the role of dopamine during LTP and LTD. *Neurobiol Learn Mem* 82(1), 12-25. doi: 10.1016/j.nlm.2004.03.003.

Sajikumar, S., and Korte, M. (2011). Metaplasticity governs compartmentalization of synaptic tagging and capture through brain-derived neurotrophic factor (BDNF) and protein kinase Mzeta (PKMzeta). *Proc Natl Acad Sci U S A* 108(6), 2551-2556. doi: 10.1073/pnas.1016849108.

Sakata, K., Martinowich, K., Woo, N.H., Schloesser, R.J., Jimenez, D.V., Ji, Y., et al. (2013). Role of activity-dependent BDNF expression in hippocampal-prefrontal cortical regulation of behavioral perseverance. *Proceedings of the National Academy of Sciences of the United States of America* 110(37), 15103-15108. doi: 10.1073/pnas.1222872110.

Sakurai, E., Maeda, T., Kaneko, S., Akaike, A., and Satoh, M. (1996). Galanin inhibits long-term potentiation at Schaffer collateral-CA1 synapses in guinea-pig hippocampal slices. *Neuroscience Letters* 212(1), 21-24. doi: https://doi.org/10.1016/0304-3940(96)12772-5.

Sala, C., Rudolph-Correia, S., and Sheng, M. (2000). Developmentally regulated NMDA receptor-dependent dephosphorylation of cAMP response element-binding protein (CREB) in hippocampal neurons. *J Neurosci* 20(10), 3529-3536. doi: 10.1523/jneurosci.20-10-03529.2000.

Salery, M., Dos Santos, M., Saint-Jour, E., Moumné, L., Pagès, C., Kappès, V., et al. (2017). Activity-Regulated Cytoskeleton-Associated Protein Accumulates in the Nucleus in Response to Cocaine and Acts as a Brake on Chromatin Remodeling and Long-Term Behavioral Alterations. *Biological Psychiatry* 81(7), 573-584. doi: https://doi.org/10.1016/j.biopsych.2016.05.025.

Salloway, S., Sperling, R., Fox, N.C., Blennow, K., Klunk, W., Raskind, M., et al. (2014). Two phase 3 trials of bapineuzumab in mild-to-moderate Alzheimer's disease. *N Engl J Med* 370(4), 322-333. doi: 10.1056/NEJMoa1304839.

Salloway, S., Sperling, R., Gilman, S., Fox, N.C., Blennow, K., Raskind, M., et al. (2009). A phase 2 multiple ascending dose trial of bapineuzumab in mild to moderate Alzheimer disease. *Neurology* 73(24), 2061-2070. doi: 10.1212/WNL.0b013e3181c67808.

Samoilova, M.V., Buldakova, S.L., Vorobjev, V.S., Sharonova, I.N., and Magazanik, L.G. (1999). The open channel blocking drug, IEM-1460, reveals functionally distinct α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate receptors in rat brain neurons. *Neuroscience* 94(1), 261-268. doi: https://doi.org/10.1016/S0306-4522(99)00326-7.

Sanderson, D.J., Gray, A., Simon, A., Taylor, A.M., Deacon, R.M., Seeburg, P.H., et al. (2007). Deletion of glutamate receptor-A (GluR-A) AMPA receptor subunits impairs one-trial spatial memory. *Behav Neurosci* 121(3), 559-569. doi: 10.1037/0735-7044.121.3.559.

Sanderson, D.J., McHugh, S.B., Good, M.A., Sprengel, R., Seeburg, P.H., Rawlins, J.N.P., et al. (2010). Spatial working memory deficits in GluA1 AMPA receptor subunit knockout mice reflect impaired short-term habituation: evidence for Wagner's dual-process memory model. *Neuropsychologia* 48(8), 2303-2315. doi: 10.1016/j.neuropsychologia.2010.03.018.

Sanderson, J.L., Gorski, J.A., and Dell'Acqua, M.L. (2016). NMDA Receptor-Dependent LTD Requires Transient Synaptic Incorporation of Ca^{2+}-Permeable AMPARs Mediated by AKAP150-Anchored PKA and Calcineurin. *Neuron* 89(5), 1000-1015. doi: 10.1016/j.neuron.2016.01.043.

Sanderson, J.L., Gorski, J.A., Gibson, E.S., Lam, P., Freund, R.K., Chick, W.S., et al. (2012). AKAP150-anchored calcineurin regulates synaptic plasticity by limiting synaptic incorporation of Ca^{2+}-permeable AMPA receptors. *J Neurosci* 32(43), 15036-15052. doi: 10.1523/jneurosci.3326-12.2012.

Sanderson, T.M. (2012). Molecular Mechanisms Involved in Depotentiation and Their Relevance to Schizophrenia. *Chonnam Medical Journal* 48(1), 1-6. doi: 10.4068/cmj.2012.48.1.1.

Sanderson, T.M., Collingridge, G.L., and Fitzjohn, S.M. (2011). Differential trafficking of AMPA receptors following activation of NMDA receptors and mGluRs. *Molecular Brain* 4(1), 30. doi: 10.1186/1756-6606-4-30.

Sangiovanni, E., Brivio, P., Dell'Agli, M., and Calabrese, F. (2017). Botanicals as Modulators of Neuroplasticity: Focus on BDNF. *Neural Plasticity* 2017, 5965371. doi: 10.1155/2017/5965371.

Sanhueza, M., Fernandez-Villalobos, G., Stein, I.S., Kasumova, G., Zhang, P., Bayer, K.U., et al. (2011). Role of the CaMKII/NMDA receptor complex in the maintenance of synaptic strength. *J Neurosci* 31(25), 9170-9178. doi: 10.1523/jneurosci.1250-11.2011.

Sardar Sinha, M., Ansell-Schultz, A., Civitelli, L., Hildesjö, C., Larsson, M., Lannfelt, L., et al. (2018). Alzheimer's disease pathology propagation by exosomes containing toxic amyloid-beta oligomers. *Acta neuropathologica* 136(1), 41-56. doi: 10.1007/s00401-018-1868-1.

Sasi, M., Vignoli, B., Canossa, M., and Blum, R. (2017). Neurobiology of local and intercellular BDNF signaling. *Pflugers Arch* 469(5-6), 593-610. doi: 10.1007/s00424-017-1964-4.

Sastry, B.R., Chirwa, S.S., May, P.B.Y., Maretić, H., Pillai, G., Kao, E.Y.H., et al. (Year). "Are Nerve Growth Factors Involved in Long-Term Synaptic Potentiation in the Hippocampus and Spatial Memory?": Springer Berlin Heidelberg), 102-105.

Satoh, J., Tabunoki, H., Ishida, T., Saito, Y., and Arima, K. (2012). Immunohistochemical characterization of γ-secretase activating protein expression in Alzheimer's disease brains. *Neuropathol Appl Neurobiol* 38(2), 132-141. doi: 10.1111/j.1365-2990.2011.01206.x.

Sattler, R., Xiong, Z., Lu, W.Y., Hafner, M., MacDonald, J.F., and Tymianski, M. (1999). Specific coupling of NMDA receptor activation to nitric oxide neurotoxicity by PSD-95 protein. *Science* 284(5421), 1845-1848. doi: 10.1126/science.284.5421.1845.

Savtchouk, I., Sun, L., Bender, C.L., Yang, Q., Szabó, G., Gasparini, S., et al. (2016). Topological Regulation of Synaptic AMPA Receptor Expression by the RNA-Binding Protein CPEB3. *Cell reports* 17(1), 86-103. doi: 10.1016/j.celrep.2016.08.094.

Scantlebury, M.H., Chun, K.C., Ma, S.C., Rho, J.M., and Kim, D.Y. (2017). Adrenocorticotropic hormone protects learning and memory function in epileptic Kcna1-null mice. *Neurosci Lett* 645, 14-18. doi: 10.1016/j.neulet.2017.02.069.

Schafe, G.E., and LeDoux, J.E. (2000). Memory consolidation of auditory pavlovian fear conditioning requires protein synthesis and protein kinase A in the amygdala. *J Neurosci* 20(18), Rc96.

Schafe, G.E., Nadel, N.V., Sullivan, G.M., Harris, A., and LeDoux, J.E. (1999). Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. *Learning & memory (Cold Spring Harbor, N.Y.)* 6(2), 97-110.

Schedin-Weiss, S., Caesar, I., Winblad, B., Blom, H., and Tjernberg, L.O. (2016). Super-resolution microscopy reveals γ-secretase at both sides of the neuronal synapse. *Acta Neuropathol Commun* 4, 29. doi: 10.1186/s40478-016-0296-5.

Scheyer, A.F., Christian, D.T., Wolf, M.E., and Tseng, K.Y. (2018). Emergence of Endocytosis-Dependent mGlu1 LTD at Nucleus Accumbens Synapses After Withdrawal From Cocaine Self-Administration. *Frontiers in Synaptic Neuroscience* 10(36). doi: 10.3389/fnsyn.2018.00036.

Scheyer, A.F., Christian, D.T., Wolf, M.E., and Tseng, K.Y. (2018). Emergence of Endocytosis-Dependent mGlu1 LTD at Nucleus Accumbens Synapses After Withdrawal From Cocaine Self-Administration. *Frontiers in Synaptic Neuroscience* 10(36). doi: 10.3389/fnsyn.2018.00036.

Scheyer, A.F., Loweth, J.A., Christian, D.T., Uejima, J., Rabei, R., Le, T., et al. (2016). AMPA Receptor Plasticity in Accumbens Core Contributes to Incubation of Methamphetamine Craving. *Biological psychiatry* 80(9), 661-670. doi: 10.1016/j.biopsych.2016.04.003.

Schinstine, M., and Iacovitti, L. (1996). Expression of Neuronal Antigens by Astrocytes Derived from EGF-Generated Neuroprogenitor Cells. *Experimental Neurology* 141(1), 67-78. doi: https://doi.org/10.1006/exnr.1996.0140.

Schlesinger, F., Tammena, D., Krampfl, K., and Bufler, J. (2005). Two mechanisms of action of the adamantane derivative IEM-1460 at human AMPA-type glutamate receptors. *British Journal of Pharmacology* 145(5), 656-663. doi: 10.1038/sj.bjp.0706233.

Schlüter, O.M., Xu, W., and Malenka, R.C. (2006). Alternative N-Terminal Domains of PSD-95 and SAP97 Govern Activity-Dependent Regulation of Synaptic AMPA Receptor Function. *Neuron* 51(1), 99-111. doi: https://doi.org/10.1016/j.neuron.2006.05.016.

Schmidt-Salzmann, C., Li, L., and Bischofberger, J. (2014). Functional properties of extrasynaptic AMPA and NMDA receptors during postnatal hippocampal neurogenesis. *The Journal of physiology* 592(1), 125-140. doi: 10.1113/jphysiol.2013.267203.

Schmitt, W.B., Deacon, R.M., Reisel, D., Sprengel, R., Seeburg, P.H., Rawlins, J.N., et al. (2004). Spatial reference memory in GluR-A-deficient mice using a novel hippocampal-dependent paddling pool escape task. *Hippocampus* 14(2), 216-223. doi: 10.1002/hipo.10168.

Schneggenburger, R., Zhou, Z., Konnerth, A., and Neher, E. (1993). Fractional contribution of calcium to the cation current through glutamate receptor channels. *Neuron* 11(1), 133-143.

Schnell, E., Sizemore, M., Karimzadegan, S., Chen, L., Bredt, D.S., and Nicoll, R.A. (2002). Direct interactions between PSD-95 and stargazin control synaptic AMPA receptor number. *Proceedings of the National Academy of Sciences* 99(21), 13902-13907. doi: 10.1073/pnas.172511199.

Schochet, T.L., Kelley, A.E., and Landry, C.F. (2005). Differential expression of arc mRNA and other plasticity-related genes induced by nicotine in adolescent rat forebrain. *Neuroscience* 135(1), 285-297. doi: 10.1016/j.neuroscience.2005.05.057.

Schroeder, B.E., and Koo, E.H. (2005). To Think or Not to Think: Synaptic Activity and Aβ Release. *Neuron* 48(6), 873-875. doi: 10.1016/j.neuron.2005.12.005.

Schuette, S.R.M., Fernández-Fernández, D., Lamla, T., Rosenbrock, H., and Hobson, S. (2016). Overexpression of Protein Kinase Mζ in the Hippocampus Enhances Long-Term Potentiation and Long-Term Contextual But Not Cued Fear Memory in Rats. *The Journal of Neuroscience* 36(15), 4313-4324. doi: 10.1523/jneurosci.3600-15.2016.

Schulz, P.E., and Fitzgibbons, J.C. (1997). Differing Mechanisms of Expression for Short- and Long-Term Potentiation. *Journal of Neurophysiology* 78(1), 321-334. doi: 10.1152/jn.1997.78.1.321.

Schulz, S., Siemer, H., Krug, M., and Höllt, V. (1999). Direct evidence for biphasic cAMP responsive element-binding protein phosphorylation during long-term potentiation in the rat dentate gyrus in vivo. *J Neurosci* 19(13), 5683-5692. doi: 10.1523/jneurosci.19-13-05683.1999.

Schuman, E., and Madison, D. (1991). A requirement for the intercellular messenger nitric oxide in long-term potentiation. *Science* 254(5037), 1503-1506. doi: 10.1126/science.1720572.

Schwenk, J., Baehrens, D., Haupt, A., Bildl, W., Boudkkazi, S., Roeper, J., et al. (2014). Regional Diversity and Developmental Dynamics of the AMPA-Receptor Proteome in the Mammalian Brain. *Neuron* 84(1), 41-54. doi: https://doi.org/10.1016/j.neuron.2014.08.044.

Scoville W.B. (1954). The Limbic Lobe in Man. *Journal of Neurosurgery* 11(1), 64-66. doi: 10.3171/jns.1954.11.1.0064.

Scoville, W.B., and Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *J Neurol Neurosurg Psychiatry* 20(1), 11-21.

Seabrook, G.R., Smith, D.W., Bowery, B.J., Easter, A., Reynolds, T., Fitzjohn, S.M., et al. (1999). Mechanisms contributing to the deficits in hippocampal synaptic plasticity in mice lacking amyloid precursor protein. *Neuropharmacology* 38(3), 349-359.

Seidenbecher, T., Balschun, D., Vogel, D., and Reymann, K.G. (1993). Neuronal transmission of hippocampal CA1 neurones is modulated by corticotropin-like intermediate lobe peptide [CLIP; ACTH(18-39)]. *Peptides* 14(6), 1221-1224. doi: 10.1016/0196-9781(93)90179-k.

Seidenman, K.J., Steinberg, J.P., Huganir, R., and Malinow, R. (2003). Glutamate Receptor Subunit 2 Serine 880 Phosphorylation Modulates Synaptic Transmission and Mediates Plasticity in CA1 Pyramidal Cells. *The Journal of Neuroscience* 23(27), 9220-9228. doi: 10.1523/jneurosci.23-27-09220.2003.

Selcher, J.C., Xu, W., Hanson, J.E., Malenka, R.C., and Madison, D.V. (2012). Glutamate receptor subunit GluA1 is necessary for long-term potentiation and synapse unsilencing, but not long-term depression in mouse hippocampus. *Brain Res* 1435, 8-14. doi: 10.1016/j.brainres.2011.11.029.

Selkoe, D.J., and Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease at 25 years. *EMBO Mol Med* 8(6), 595-608. doi: 10.15252/emmm.201606210.

Selnes, P., Blennow, K., Zetterberg, H., Grambaite, R., Rosengren, L., Johnsen, L., et al. (2010). Effects of cerebrovascular disease on amyloid precursor protein metabolites in cerebrospinal fluid. *Cerebrospinal Fluid Res* 7, 10. doi: 10.1186/1743-8454-7-10.

Semenov, A., Möykkynen, T., Coleman, S.K., Korpi, E.R., and Keinänen, K. (2012). Autoinactivation of the Stargazin–AMPA Receptor Complex: Subunit-Dependency and Independence from Physical Dissociation. *PLOS ONE* 7(11), e49282. doi: 10.1371/journal.pone.0049282.

Semon, R.W. (1911). *Die Mneme als erhaltendes Prinzip im Wechsel des organischen Geschehens.* Engelmann.

Sennvik, K., Fastbom, J., Blomberg, M., Wahlund, L.-O., Winblad, B., and Benedikz, E. (2000). Levels of α- and β-secretase cleaved amyloid precursor protein in the cerebrospinal fluid of Alzheimer's disease patients. *Neuroscience Letters* 278(3), 169-172. doi: https://doi.org/10.1016/S0304-3940(99)00929-5.

Seo, S.Y., Oh, J.H., and Choe, E.S. (2013). Protein kinase G increases AMPA receptor GluR1 phosphorylation at serine 845 after repeated cocaine administration in the rat nucleus accumbens. *Neurosci Lett* 544, 147-151. doi: 10.1016/j.neulet.2013.04.003.

Serrano, P., Friedman, E.L., Kenney, J., Taubenfeld, S.M., Zimmerman, J.M., Hanna, J., et al. (2008). PKMzeta maintains spatial, instrumental, and classically conditioned long-term memories. *PLoS biology* 6(12), 2698-2706. doi: 10.1371/journal.pbio.0060318.

Serulle, Y., Zhang, S., Ninan, I., Puzzo, D., McCarthy, M., Khatri, L., et al. (2007). A GluR1-cGKII interaction regulates AMPA receptor trafficking. *Neuron* 56(4), 670-688. doi: 10.1016/j.neuron.2007.09.016.

Sevigny, J., Chiao, P., Bussiere, T., Weinreb, P.H., Williams, L., Maier, M., et al. (2016). The antibody aducanumab reduces Abeta plaques in Alzheimer's disease. *Nature* 537(7618), 50-56. doi: 10.1038/nature19323.

Seyfarth, R.M., and Cheney, D.L. (2003). Signalers and receivers in animal communication. *Annu Rev Psychol* 54, 145-173. doi: 10.1146/annurev.psych.54.101601.145121.

Sgambato-Faure, V., Buggia, V., Gilbert, F., Lévesque, D., Benabid, A.-L., and Berger, F. (2005). Coordinated and Spatial Upregulation of Arc in Striatonigral Neurons Correlates With L-Dopa-Induced Behavioral Sensitization in Dyskinetic Rats. *Journal of Neuropathology & Experimental Neurology* 64(11), 936-947. doi: 10.1097/01.jnen.0000186922.42592.b7.

Shankar, G.M., Li, S., Mehta, T.H., Garcia-Munoz, A., Shepardson, N.E., Smith, I., et al. (2008). Amyloid-beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nature medicine* 14(8), 837-842. doi: 10.1038/nm1782.

Sharma, G., and Vijayaraghavan, S. (2003). Modulation of Presynaptic Store Calcium Induces Release of Glutamate and Postsynaptic Firing. *Neuron* 38(6), 929-939. doi: https://doi.org/10.1016/S0896-6273(03)00322-2.

Sharma, G., Grybko, M., and Vijayaraghavan, S. (2008). Action Potential-Independent and Nicotinic Receptor-Mediated Concerted Release of Multiple Quanta at Hippocampal CA3–Mossy Fiber Synapses. *The Journal of Neuroscience* 28(10), 2563-2575. doi: 10.1523/jneurosci.5407-07.2008.

Shashoua, V.E. (1976). Identification of specific changes in the pattern of brain protein synthesis after training. *Science* 193(4259), 1264-1266.

Shen, F., Li, Y.J., Shou, X.J., and Cui, C.L. (2012). Role of the NO/sGC/PKG signaling pathway of hippocampal CA1 in morphine-induced reward memory. *Neurobiol Learn Mem* 98(2), 130-138. doi: 10.1016/j.nlm.2012.07.005.

Shen, F., Wang, N., Qi, C., Li, Y.J., and Cui, C.L. (2014). The NO/sGC/PKG signaling pathway in the NAc shell is necessary for the acquisition of morphine-induced place preference. *Behav Neurosci* 128(4), 446-459. doi: 10.1037/a0036964.

Shen, F., Wang, X.W., Ge, F.F., Li, Y.J., and Cui, C.L. (2016). Essential role of the NO signaling pathway in the hippocampal CA1 in morphine-associated memory depends on glutaminergic receptors. *Neuropharmacology* 102, 216-228. doi: 10.1016/j.neuropharm.2015.11.008.

Shen, L., Cui, W.Y., Chen, R.Z., and Wang, H. (2016). Differential modulation of GABAA and NMDA receptors by α7-nicotinic receptor desensitization in cultured rat hippocampal neurons. *Acta Pharmacol Sin* 37(3), 312-321. doi: 10.1038/aps.2015.106.

Shen, L., Liang, F., Walensky, L.D., and Huganir, R.L. (2000). Regulation of AMPA receptor GluR1 subunit surface expression by a 4. 1N-linked actin cytoskeletal association. *J Neurosci* 20(21), 7932-7940. doi: 10.1523/jneurosci.20-21-07932.2000.

Sheng, M., Cummings, J., Roldan, L.A., Jan, Y.N., and Jan, L.Y. (1994). Changing subunit composition of heteromeric NMDA receptors during development of rat cortex. *Nature* 368(6467), 144-147. doi: 10.1038/368144a0.

Sheng, M., Thompson, M., and Greenberg, M. (1991). CREB: a Ca^{2+}-regulated transcription factor phosphorylated by calmodulin-dependent kinases. *Science* 252(5011), 1427-1430. doi: 10.1126/science.1646483.

Shepherd, J.D. (2012). Memory, plasticity and sleep - A role for calcium permeable AMPA receptors? *Frontiers in molecular neuroscience* 5, 49-49. doi: 10.3389/fnmol.2012.00049.

Shepherd, J.D., and Bear, M.F. (2011). New views of Arc, a master regulator of synaptic plasticity. *Nature Neuroscience* 14(3), 279-284. doi: 10.1038/nn.2708.
Shepherd, J.D., Rumbaugh, G., Wu, J., Chowdhury, S., Plath, N., Kuhl, D., et al. (2006). Arc/Arg3.1 mediates homeostatic synaptic scaling of AMPA receptors. *Neuron* 52(3), 475-484. doi: 10.1016/j.neuron.2006.08.034.
Sherry, D.F., and Schacter, D.L. (1987). The evolution of multiple memory systems. *Psychological review* 94(4), 439.
Shetty, M.S., Sharma, M., and Sajikumar, S. (2017). Chelation of hippocampal zinc enhances long-term potentiation and synaptic tagging/capture in CA1 pyramidal neurons of aged rats: implications to aging and memory. *Aging Cell* 16(1), 136-148. doi: 10.1111/acel.12537.
Sheynikhovich, D., Otani, S., and Arleo, A. (2013). Dopaminergic control of long-term depression/long-term potentiation threshold in prefrontal cortex. *J Neurosci* 33(34), 13914-13926. doi: 10.1523/jneurosci.0466-13.2013.
Shi, M., Majumdar, D., Gao, Y., Brewer, B.M., Goodwin, C.R., McLean, J.A., et al. (2013). Glia co-culture with neurons in microfluidic platforms promotes the formation and stabilization of synaptic contacts. *Lab Chip* 13(15), 3008-3021. doi: 10.1039/c3lc50249j.
Shi, S.-H., Hayashi, Y., Esteban, J.A., and Malinow, R. (2001). Subunit-Specific Rules Governing AMPA Receptor Trafficking to Synapses in Hippocampal Pyramidal Neurons. *Cell* 105(3), 331-343. doi: https://doi.org/10.1016/S0092-8674(01)00321-X.
Shi, S.-H., Hayashi, Y., Petralia, R.S., Zaman, S.H., Wenthold, R.J., Svoboda, K., et al. (1999). Rapid Spine Delivery and Redistribution of AMPA Receptors After Synaptic NMDA Receptor Activation. *Science* 284(5421), 1811-1816. doi: 10.1126/science.284.5421.1811.
Shi, Y., Suh, Y.H., Milstein, A.D., Isozaki, K., Schmid, S.M., Roche, K.W., et al. (2010). Functional comparison of the effects of TARPs and cornichons on AMPA receptor trafficking and gating. *Proceedings of the National Academy of Sciences* 107(37), 16315-16319. doi: 10.1073/pnas.1011706107.
Shimshek, D.R., Jensen, V., Celikel, T., Geng, Y., Schupp, B., Bus, T., et al. (2006). Forebrain-specific glutamate receptor B deletion impairs spatial memory but not hippocampal field long-term potentiation. *J Neurosci* 26(33), 8428-8440. doi: 10.1523/jneurosci.5410-05.2006.
Shinohara, Y., and Hirase, H. (2009). Size and Receptor Density of Glutamatergic Synapses: A Viewpoint from Left-Right Asymmetry of CA3-CA1 Connections. *Frontiers in neuroanatomy* 3, 10-10. doi: 10.3389/neuro.05.010.2009.
Shors, T.J., and Matzel, L.D. (1997). Long-term potentiation: what's learning got to do with it? *Behav Brain Sci* 20(4), 597-614; discussion 614-555. doi: 10.1017/s0140525x97001593.
Si, K., Giustetto, M., Etkin, A., Hsu, R., Janisiewicz, A.M., Miniaci, M.C., et al. (2003). A neuronal isoform of CPEB regulates local protein synthesis and stabilizes synapse-specific long-term facilitation in aplysia. *Cell* 115(7), 893-904. doi: 10.1016/s0092-8674(03)01021-3.
Silva, A., Paylor, R., Wehner, J., and Tonegawa, S. (1992a). Impaired spatial learning in alpha-calcium-calmodulin kinase II mutant mice. *Science* 257(5067), 206-211. doi: 10.1126/science.1321493.
Silva, A.J., Stevens, C.F., Tonegawa, S., and Wang, Y. (1992b). Deficient Hippocampal Long-Term Potentiation in α-Calcium-Calmodulin Kinase II Mutant Mice. *Science* 257(5067), 201-206.
Silverman, J.B., Restituito, S., Lu, W., Lee-Edwards, L., Khatri, L., and Ziff, E.B. (2007). Synaptic anchorage of AMPA receptors by cadherins through neural plakophilin-related arm protein AMPA receptor-binding protein complexes. *J Neurosci* 27(32), 8505-8516. doi: 10.1523/jneurosci.1395-07.2007.
Silverstein, J. (2012). Mapping the Motor and Sensory Cortices: A Historical Look and a Current Case Study in Sensorimotor Localization and Direct Cortical Motor Stimulation. *The Neurodiagnostic Journal* 52(1), 54-68. doi: 10.1080/21646821.2012.11079843.

Singh, A., Jones, O.D., Mockett, B.G., Ohline, S.M., and Abraham, W.C. (2019). Tumor Necrosis Factor-α-Mediated Metaplastic Inhibition of LTP Is Constitutively Engaged in an Alzheimer's Disease Model. *J Neurosci* 39(46), 9083-9097. doi: 10.1523/jneurosci.1492-19.2019.

Siopi, E., Llufriu-Daben, G., Cho, A.H., Vidal-Lletjos, S., Plotkine, M., Marchand-Leroux, C., et al. (2013). Etazolate, an alpha-secretase activator, reduces neuroinflammation and offers persistent neuroprotection following traumatic brain injury in mice. *Neuropharmacology* 67, 183-192. doi: 10.1016/j.neuropharm.2012.11.009.

Sjostrom, P.J., Turrigiano, G.G., and Nelson, S.B. (2007). Multiple forms of long-term plasticity at unitary neocortical layer 5 synapses. *Neuropharmacology* 52(1), 176-184. doi: 10.1016/j.neuropharm.2006.07.021.

Skålhegg, B.S., Huang, Y., Su, T., Idzerda, R.L., McKnight, G.S., and Burton, K.A. (2002). Mutation of the Cα Subunit of PKA Leads to Growth Retardation and Sperm Dysfunction. *Molecular Endocrinology* 16(3), 630-639. doi: 10.1210/mend.16.3.0793.

Skeberdis, V.A., Chevaleyre, V., Lau, C.G., Goldberg, J.H., Pettit, D.L., Suadicani, S.O., et al. (2006). Protein kinase A regulates calcium permeability of NMDA receptors. *Nat Neurosci* 9(4), 501-510. doi: 10.1038/nn1664.

Skovronsky, D.M., Moore, D.B., Milla, M.E., Doms, R.W., and Lee, V.M. (2000). Protein kinase C-dependent alpha-secretase competes with beta-secretase for cleavage of amyloid-beta precursor protein in the trans-golgi network. *J Biol Chem* 275(4), 2568-2575. doi: 10.1074/jbc.275.4.2568.

Small, D.H., Nurcombe, V., Reed, G., Clarris, H., Moir, R., Beyreuther, K., et al. (1994). A heparin-binding domain in the amyloid protein precursor of Alzheimer's disease is involved in the regulation of neurite outgrowth. *J Neurosci* 14(4), 2117-2127.

Smith-Swintosky, V.L., Pettigrew, L.C., Craddock, S.D., Culwell, A.R., Rydel, R.E., and Mattson, M.P. (1994). Secreted forms of beta-amyloid precursor protein protect against ischemic brain injury. *J Neurochem* 63(2), 781-784. doi: 10.1046/j.1471-4159.1994.63020781.x.

Smith, B.A., Daugherty-Clarke, K., Goode, B.L., and Gelles, J. (2013). Pathway of actin filament branch formation by Arp2/3 complex revealed by single-molecule imaging. *Proceedings of the National Academy of Sciences of the United States of America* 110(4), 1285-1290. doi: 10.1073/pnas.1211164110.

Smith, K.E., Gibson, E.S., and Dell'Acqua, M.L. (2006). cAMP-Dependent Protein Kinase Postsynaptic Localization Regulated by NMDA Receptor Activation through Translocation of an A-Kinase Anchoring Protein Scaffold Protein. *The Journal of Neuroscience* 26(9), 2391-2402. doi: 10.1523/jneurosci.3092-05.2006.

Smith, W.B., Starck, S.R., Roberts, R.W., and Schuman, E.M. (2005). Dopaminergic Stimulation of Local Protein Synthesis Enhances Surface Expression of GluR1 and Synaptic Transmission in Hippocampal Neurons. *Neuron* 45(5), 765-779. doi: https://doi.org/10.1016/j.neuron.2005.01.015.

Snyder, E.M., Nong, Y., Almeida, C.G., Paul, S., Moran, T., Choi, E.Y., et al. (2005). Regulation of NMDA receptor trafficking by amyloid-beta. *Nat Neurosci* 8(8), 1051-1058. doi: 10.1038/nn1503.

Soares, C., Lee, K.F., Nassrallah, W., and Beique, J.C. (2013). Differential subcellular targeting of glutamate receptor subtypes during homeostatic synaptic plasticity. *J Neurosci* 33(33), 13547-13559. doi: 10.1523/jneurosci.1873-13.2013.

Sofroniew, M.V., and Vinters, H.V. (2010). Astrocytes: biology and pathology. *Acta neuropathologica* 119(1), 7-35. doi: 10.1007/s00401-009-0619-8.

Soldati, T., and Schliwa, M. (2006). Powering membrane traffic in endocytosis and recycling. *Nat Rev Mol Cell Biol* 7(12), 897-908. doi: 10.1038/nrm2060.

Solinas, S.M.G., Edelmann, E., Leßmann, V., and Migliore, M. (2019). A kinetic model for Brain-Derived Neurotrophic Factor mediated spike timing-dependent LTP. *PLOS Computational Biology* 15(4), e1006975. doi: 10.1371/journal.pcbi.1006975.

Son, H., Lu, Y.F., Zhuo, M., Arancio, O., Kandel, E.R., and Hawkins, R.D. (1998). The specific role of cGMP in hippocampal LTP. *Learning & memory (Cold Spring Harbor, N.Y.)* 5(3), 231-245.

Sossin, W., Heaney, C.F., and Raab-Graham, K.F. (2019). "Dysregulated Protein Synthesis in Major Depressive Disorder". Oxford University Press).

Soto, D., Coombs, I.D., Gratacòs-Batlle, E., Farrant, M., and Cull-Candy, S.G. (2014). Molecular mechanisms contributing to TARP regulation of channel conductance and polyamine block of calcium-permeable AMPA receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 34(35), 11673-11683. doi: 10.1523/JNEUROSCI.0383-14.2014.

Soulé, J., Alme, M., Myrum, C., Schubert, M., Kanhema, T., and Bramham, C.R. (2012). Balancing Arc synthesis, mRNA decay, and proteasomal degradation: maximal protein expression triggered by rapid eye movement sleep-like bursts of muscarinic cholinergic receptor stimulation. *J Biol Chem* 287(26), 22354-22366. doi: 10.1074/jbc.M112.376491.

Soumier, A., Lortet, S., Masmejean, F., Gabriel, C., Mocaer, E., Millan, M., et al. (2009). P.2.d.018 Mechanisms contributing to the regulation of the different phases of neurogenesis by agomelatine. *European Neuropsychopharmacology* 19, S439-S440. doi: https://doi.org/10.1016/S0924-977X(09)70684-4.

Southam, K.A., Stennard, F., Pavez, C., and Small, D.H. (2019). Knockout of Amyloid β Protein Precursor (APP) Expression Alters Synaptogenesis, Neurite Branching and Axonal Morphology of Hippocampal Neurons. *Neurochemical Research* 44(6), 1346-1355. doi: 10.1007/s11064-018-2512-0.

Spillantini, M.G., Goedert, M., Jakes, R., and Klug, A. (1990). Different configurational states of beta-amyloid and their distributions relative to plaques and tangles in Alzheimer disease. *Proceedings of the National Academy of Sciences* 87(10), 3947-3951. doi: 10.1073/pnas.87.10.3947.

Spires-Jones, T.L., Meyer-Luehmann, M., Osetek, J.D., Jones, P.B., Stern, E.A., Bacskai, B.J., et al. (2007). Impaired spine stability underlies plaque-related spine loss in an Alzheimer's disease mouse model. *Am J Pathol* 171(4), 1304-1311. doi: 10.2353/ajpath.2007.070055.

Squire, L.R. (2009). The Legacy of Patient H.M. for Neuroscience. *Neuron* 61(1), 6-9. doi: 10.1016/j.neuron.2008.12.023.

Squire, L.R., and Zola-Morgan, S. (1991). The medial temporal lobe memory system. *Science* 253(5026), 1380-1386.

Stamford, A.W., Scott, J.D., Li, S.W., Babu, S., Tadesse, D., Hunter, R., et al. (2012). Discovery of an Orally Available, Brain Penetrant BACE1 Inhibitor that Affords Robust CNS Abeta Reduction. *ACS Med Chem Lett* 3(11), 897-902. doi: 10.1021/ml3001165.

Statello, L., Maugeri, M., Garre, E., Nawaz, M., Wahlgren, J., Papadimitriou, A., et al. (2018). Identification of RNA-binding proteins in exosomes capable of interacting with different types of RNA: RBP-facilitated transport of RNAs into exosomes. *PloS one* 13(4), e0195969-e0195969. doi: 10.1371/journal.pone.0195969.

Stein, T.D., Anders, N.J., DeCarli, C., Chan, S.L., Mattson, M.P., and Johnson, J.A. (2004). Neutralization of Transthyretin Reverses the Neuroprotective Effects of Secreted Amyloid Precursor Protein (APP) in APP_{Sw} Mice Resulting in Tau Phosphorylation and Loss of Hippocampal Neurons: Support for the Amyloid Hypothesis. *The Journal of Neuroscience* 24(35), 7707-7717. doi: 10.1523/jneurosci.2211-04.2004.

Stein, V., House, D.R.C., Bredt, D.S., and Nicoll, R.A. (2003). Postsynaptic Density-95 Mimics and Occludes Hippocampal Long-Term Potentiation and Enhances Long-Term Depression. *The Journal of Neuroscience* 23(13), 5503-5506. doi: 10.1523/jneurosci.23-13-05503.2003.

Steinacker, P., Fang, L., Kuhle, J., Petzold, A., Tumani, H., Ludolph, A.C., et al. (2011). Soluble Beta-Amyloid Precursor Protein Is Related to Disease Progression in Amyotrophic Lateral Sclerosis. *PLOS ONE* 6(8), e23600. doi: 10.1371/journal.pone.0023600.

Steinvorth, S., Levine, B., and Corkin, S. (2005). Medial temporal lobe structures are needed to re-experience remote autobiographical memories: evidence from H.M. and W.R. *Neuropsychologia* 43(4), 479-496. doi: 10.1016/j.neuropsychologia.2005.01.001.

Stellwagen, D., and Malenka, R.C. (2006). Synaptic scaling mediated by glial TNF-alpha. *Nature* 440(7087), 1054-1059. doi: 10.1038/nature04671.

Stephenson, F.A. (2001). Subunit characterization of NMDA receptors. *Curr Drug Targets* 2(3), 233-239. doi: 10.2174/1389450013348461.

Stevens, C.F., and Sullivan, J.M. (2003). The synaptotagmin C2A domain is part of the calcium sensor controlling fast synaptic transmission. *Neuron* 39(2), 299-308.

Steward, O., and Levy, W.B. (1982). Preferential localization of polyribosomes under the base of dendritic spines in granule cells of the dentate gyrus. *Journal of Neuroscience* 2(3), 284-291.

Steward, O., and Schuman, E.M. (2001). Protein synthesis at synaptic sites on dendrites. *Annu Rev Neurosci* 24, 299-325. doi: 10.1146/annurev.neuro.24.1.299.

Steward, O., and Worley, P.F. (2001). A cellular mechanism for targeting newly synthesized mRNAs to synaptic sites on dendrites. *Proc Natl Acad Sci U S A* 98(13), 7062-7068. doi: 10.1073/pnas.131146398.

Steward, O., Farris, S., Pirbhoy, P.S., Darnell, J., and Driesche, S.J.V. (2015). Localization and local translation of Arc/Arg3.1 mRNA at synapses: some observations and paradoxes. *Frontiers in Molecular Neuroscience* 7(101). doi: 10.3389/fnmol.2014.00101.

Steward, O., Matsudaira Yee, K., Farris, S., Pirbhoy, P.S., Worley, P., Okamura, K., et al. (2018). Delayed Degradation and Impaired Dendritic Delivery of Intron-Lacking EGFP-Arc/Arg3.1 mRNA in EGFP-Arc Transgenic Mice. *Frontiers in molecular neuroscience* 10, 435-435. doi: 10.3389/fnmol.2017.00435.

Steward, O., Wallace, C.S., Lyford, G.L., and Worley, P.F. (1998). Synaptic Activation Causes the mRNA for the IEG Arc to Localize Selectively near Activated Postsynaptic Sites on Dendrites. *Neuron* 21(4), 741-751. doi: 10.1016/S0896-6273(00)80591-7.

Stoop, R., and Poo, M.M. (1996). Synaptic modulation by neurotrophic factors: differential and synergistic effects of brain-derived neurotrophic factor and ciliary neurotrophic factor. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 16(10), 3256-3264. doi: 10.1523/JNEUROSCI.16-10-03256.1996.

Strack, S., Choi, S., Lovinger, D.M., and Colbran, R.J. (1997). Translocation of Autophosphorylated Calcium/Calmodulin-dependent Protein Kinase II to the Postsynaptic Density. *Journal of Biological Chemistry* 272(21), 13467-13470. doi: 10.1074/jbc.272.21.13467.

Strack, S., McNeill, R.B., and Colbran, R.J. (2000). Mechanism and Regulation of Calcium/Calmodulin-dependent Protein Kinase II Targeting to the NR2B Subunit of the N-Methyl-d-aspartate Receptor. *Journal of Biological Chemistry* 275(31), 23798-23806. doi: 10.1074/jbc.M001471200.

Strange, B.A., Witter, M.P., Lein, E.S., and Moser, E.I. (2014). Functional organization of the hippocampal longitudinal axis. *Nature Reviews Neuroscience* 15(10), 655-669. doi: 10.1038/nrn3785.

Stricker, C., Field, A.C., and Redman, S.J. (1996). Changes in quantal parameters of EPSCs in rat CA1 neurones in vitro after the induction of long-term potentiation. *J Physiol* 490 (Pt 2), 443-454. doi: 10.1113/jphysiol.1996.sp021156.

Struyfs, H., Van Broeck, B., Timmers, M., Fransen, E., Sleegers, K., Van Broeckhoven, C., et al. (2015). Diagnostic Accuracy of Cerebrospinal Fluid Amyloid-β Isoforms for Early and Differential Dementia Diagnosis. *J Alzheimers Dis* 45(3), 813-822. doi: 10.3233/jad-141986.

Summers, K.C., Bogard, A.S., and Tavalin, S.J. (2019). Preferential generation of Ca^{2+}-permeable AMPA receptors by AKAP79-anchored protein kinase C proceeds via GluA1 subunit phosphorylation at Ser-831. *Journal of Biological Chemistry* 294(14), 5521-5535. doi: 10.1074/jbc.RA118.004340.

Sun, X., Hu, X., Wang, D., Yuan, Y., Qin, S., Tan, Z., et al. (2017). Establishment and characterization of primary astrocyte culture from adult mouse brain. *Brain Research Bulletin* 132, 10-19. doi: https://doi.org/10.1016/j.brainresbull.2017.05.002.

Sundborger, A., Soderblom, C., Vorontsova, O., Evergren, E., Hinshaw, J.E., and Shupliakov, O. (2011). An endophilin-dynamin complex promotes budding of clathrin-coated vesicles during synaptic vesicle recycling. *Journal of cell science* 124(Pt 1), 133-143. doi: 10.1242/jcs.072686.

Suske, G. (1999). The Sp-family of transcription factors. *Gene* 238(2), 291-300. doi: https://doi.org/10.1016/S0378-1119(99)00357-1.

Sutherland, E.W., and Rall, T.W. (1958). Fractionation and characterization of a cyclic adenine ribonucleotide formed by tissue particles. *J Biol Chem* 232(2), 1077-1091.

Sutton, M.A., and Schuman, E.M. (2006). Dendritic Protein Synthesis, Synaptic Plasticity, and Memory. *Cell* 127(1), 49-58. doi: https://doi.org/10.1016/j.cell.2006.09.014.

Sutton, M.A., Ito, H.T., Cressy, P., Kempf, C., Woo, J.C., and Schuman, E.M. (2006). Miniature neurotransmission stabilizes synaptic function via tonic suppression of local dendritic protein synthesis. *Cell* 125(4), 785-799. doi: 10.1016/j.cell.2006.03.040.

Suyama, S., Ralevski, A., Liu, Z.-W., Dietrich, M.O., Yada, T., Simonds, S.E., et al. (2017). Plasticity of calcium-permeable AMPA glutamate receptors in Pro-opiomelanocortin neurons. *eLife* 6, e25755. doi: 10.7554/eLife.25755.

Suzuki, T. (1994). Protein kinases involved in the expression of long-term potentiation. *Int J Biochem* 26(6), 735-744.

Swant, J., and Wagner, J.J. (2006). Dopamine transporter blockade increases LTP in the CA1 region of the rat hippocampus via activation of the D3 dopamine receptor. *Learning & memory (Cold Spring Harbor, N.Y.)* 13(2), 161-167. doi: 10.1101/lm.63806.

Szczot, M., Wojtowicz, T., and Mozrzymas, J.W. (2010). GABAergic and glutamatergic currents in hippocampal slices and neuronal cultures show profound differences: a clue to a potent homeostatic modulation. J Physiol Pharmacol 61(4), 501-506.

Tackenberg, C., and Nitsch, R.M. (2019). The secreted APP ectodomain sAPPα, but not sAPPβ, protects neurons against Aβ oligomer-induced dendritic spine loss and increased tau phosphorylation. *Molecular Brain* 12(1), 27. doi: 10.1186/s13041-019-0447-2.

Tada, H., Koide, M., Ara, W., Shibata, Y., Funabashi, T., Suyama, K., et al. (2015). Estrous Cycle-Dependent Phasic Changes in the Stoichiometry of Hippocampal Synaptic AMPA Receptors in Rats. *PLOS ONE* 10(6), e0131359. doi: 10.1371/journal.pone.0131359.

Tada, H., Kuroki, Y., Funabashi, T., Kamiya, Y., Goto, T., Suyama, K., et al. (2013). Phasic synaptic incorporation of GluR2-lacking AMPA receptors at gonadotropin-releasing hormone neurons is involved in the generation of the luteinizing hormone surge in female rats. *Neuroscience* 248, 664-669. doi: 10.1016/j.neuroscience.2013.06.040.

Takada-Takatori, Y., Nakagawa, S., Kimata, R., Nao, Y., Mizukawa, Y., Urushidani, T., et al. (2019). Donepezil modulates amyloid precursor protein endocytosis and reduction by up-regulation of SNX33 expression in primary cortical neurons. *Sci Rep* 9(1), 11922. doi: 10.1038/s41598-019-47462-4.

Takahashi, T., Svoboda, K., and Malinow, R. (2003). Experience Strengthening Transmission by Driving AMPA Receptors into Synapses. *Science* 299(5612), 1585-1588. doi: 10.1126/science.1079886.

Takao, K., Okamoto, K., Nakagawa, T., Neve, R.L., Nagai, T., Miyawaki, A., et al. (2005). Visualization of synaptic Ca^{2+}/calmodulin-dependent protein kinase II activity in living neurons. *J Neurosci* 25(12), 3107-3112. doi: 10.1523/jneurosci.0085-05.2005.

Takemoto, K., Iwanari, H., Tada, H., Suyama, K., Sano, A., Nagai, T., et al. (2017). Optical inactivation of synaptic AMPA receptors erases fear memory. *Nature Biotechnology* 35(1), 38-47. doi: 10.1038/nbt.3710.

Takkala, P., and Woodin, M.A. (2013). Muscarinic acetylcholine receptor activation prevents disinhibition-mediated LTP in the hippocampus. *Frontiers in cellular neuroscience* 7, 16-16. doi: 10.3389/fncel.2013.00016.

Tan, V.T.Y., Mockett, B.G., Ohline, S.M., Parfitt, K.D., Wicky, H.E., Peppercorn, K., et al. (2018). Lentivirus-mediated expression of human secreted amyloid precursor protein-alpha prevents development of memory and plasticity deficits in a mouse model of Alzheimer's disease. *Molecular brain* 11(1), 7-7. doi: 10.1186/s13041-018-0348-9.

Tanaka, H., and Hirano, T. (2012). Visualization of subunit-specific delivery of glutamate receptors to postsynaptic membrane during hippocampal long-term potentiation. *Cell Rep* 1(4), 291-298. doi: 10.1016/j.celrep.2012.02.004.

Tanaka, S., Shiojiri, S., Takahashi, Y., Kitaguchi, N., Ito, H., Kameyama, M., et al. (1989). Tissue-specific expression of three types of β-protein precursor mRNA: Enhancement of protease inhibitor-harboring types in Alzheimer's disease brain. *Biochemical and Biophysical Research Communications* 165(3), 1406-1414. doi: https://doi.org/10.1016/0006-291X(89)92760-5.

Tang, B., Luo, D., Yang, J., Xu, X.-Y., Zhu, B.-L., Wang, X.-F., et al. (2015). Modulation of AMPA receptor mediated current by nicotinic acetylcholine receptor in layer I neurons of rat prefrontal cortex. *Scientific reports* 5, 14099-14099. doi: 10.1038/srep14099.

Tang, X., Li, Y., Ao, J., Ding, L., Liu, Y., Yuan, Y., et al. (2018). Role of α7nAChR-NMDAR in sevoflurane-induced memory deficits in the developing rat hippocampus. *PloS one* 13(2), e0192498-e0192498. doi: 10.1371/journal.pone.0192498.

Taniguchi, T., Kawamata, T., Mukai, H., Hasegawa, H., Isagawa, T., Yasuda, M., et al. (2001). Phosphorylation of tau is regulated by PKN. *J Biol Chem* 276(13), 10025-10031. doi: 10.1074/jbc.M007427200.

Tanzi, E. (1893). I fatti e le induzioni dell'odierna istologia del sistema nervoso. *Rivista Sperimentale di Freniatria* 19, 419.

Tao-Cheng, J.H., Crocker, V.T., Winters, C.A., Azzam, R., Chludzinski, J., and Reese, T.S. (2011). Trafficking of AMPA receptors at plasma membranes of hippocampal neurons. *J Neurosci* 31(13), 4834-4843. doi: 10.1523/jneurosci.4745-10.2011.

Tardin, C., Cognet, L., Bats, C., Lounis, B., and Choquet, D. (2003). Direct imaging of lateral movements of AMPA receptors inside synapses. *The EMBO Journal* 22(18), 4656-4665. doi: 10.1093/emboj/cdg463.

Tartaglia, N., Du, J., Tyler, W.J., Neale, E., Pozzo-Miller, L., and Lu, B. (2001). Protein Synthesis-dependent and -independent Regulation of Hippocampal Synapses by Brain-derived Neurotrophic Factor. *Journal of Biological Chemistry* 276(40), 37585-37593. doi: 10.1074/jbc.M101683200.

Tavalin, S.J. (2008). AKAP79 selectively enhances protein kinase C regulation of GluR1 at a Ca^{2+}-calmodulin-dependent protein kinase II/protein kinase C site. *J Biol Chem* 283(17), 11445-11452. doi: 10.1074/jbc.M709253200.

Tavalin, S.J., Colledge, M., Hell, J.W., Langeberg, L.K., Huganir, R.L., and Scott, J.D. (2002a). Regulation of GluR1 by the A-Kinase Anchoring Protein 79 (AKAP79) Signaling Complex Shares Properties with Long-Term Depression. *The Journal of Neuroscience* 22(8), 3044-3051. doi: 10.1523/jneurosci.22-08-03044.2002.

Taylor, A.M., Dieterich, D.C., Ito, H.T., Kim, S.A., and Schuman, E.M. (2010). Microfluidic local perfusion chambers for the visualization and manipulation of synapses. *Neuron* 66(1), 57-68. doi: 10.1016/j.neuron.2010.03.022.

Taylor, C.J., Ireland, D.R., Ballagh, I., Bourne, K., Marechal, N.M., Turner, P.R., et al. (2008). Endogenous secreted amyloid precursor protein-α regulates hippocampal NMDA receptor function, long-term potentiation and spatial memory. *Neurobiology of Disease* 31(2), 250-260. doi: https://doi.org/10.1016/j.nbd.2008.04.011.

Terashima, A., Cotton, L., Dev, K.K., Meyer, G., Zaman, S., Duprat, F., et al. (2004). Regulation of synaptic strength and AMPA receptor subunit composition by PICK1. *J Neurosci* 24(23), 5381-5390. doi: 10.1523/jneurosci.4378-03.2004.

Terashima, A., Suh, Y.H., and Isaac, J.T.R. (2017). The AMPA Receptor Subunit GluA1 is Required for CA1 Hippocampal Long-Term Potentiation but is not Essential for Synaptic Transmission. *Neurochem Res*. doi: 10.1007/s11064-017-2425-3.

Terunuma, M., Revilla-Sanchez, R., Quadros, I.M., Deng, Q., Deeb, T.Z., Lumb, M., et al. (2014). Postsynaptic GABAB receptor activity regulates excitatory neuronal architecture and spatial memory. *J Neurosci* 34(3), 804-816. doi: 10.1523/jneurosci.3320-13.2013.

Teyler, T.J. (1999). Use of brain slices to study long-term potentiation and depression as examples of synaptic plasticity. *Methods* 18(2), 109-116. doi: 10.1006/meth.1999.0764.

Thalhammer, A., Rudhard, Y., Tigaret, C.M., Volynski, K.E., Rusakov, D.A., and Schoepfer, R. (2006). CaMKII translocation requires local NMDA receptor-mediated Ca^{2+} signaling. *Embo j* 25(24), 5873-5883. doi: 10.1038/sj.emboj.7601420.

Thiagarajan, T.C., Lindskog, M., and Tsien, R.W. (2005). Adaptation to synaptic inactivity in hippocampal neurons. *Neuron* 47(5), 725-737. doi: 10.1016/j.neuron.2005.06.037.

Thordardottir, S., Kinhult Ståhlbom, A., Almkvist, O., Thonberg, H., Eriksdotter, M., Zetterberg, H., et al. (2017). The effects of different familial Alzheimer's disease mutations on APP processing in vivo. *Alzheimers Res Ther* 9. doi: 10.1186/s13195-017-0234-1.

Thornton, E., Vink, R., Blumbergs, P.C., and Van Den Heuvel, C. (2006). Soluble amyloid precursor protein alpha reduces neuronal injury and improves functional outcome following diffuse traumatic brain injury in rats. *Brain Res* 1094(1), 38-46. doi: 10.1016/j.brainres.2006.03.107.

Timofeeva, Y., and Volynski, K.E. (2015). Calmodulin as a major calcium buffer shaping vesicular release and short-term synaptic plasticity: facilitation through buffer dislocation. *Frontiers in Cellular Neuroscience* 9, 239. doi: 10.3389/fncel.2015.00239.

Todd, G.K., Boosalis, C.A., Burzycki, A.A., Steinman, M.Q., Hester, L.D., Shuster, P.W., et al. (2013). Towards Neuronal Organoids: A Method for Long-Term Culturing of High-Density Hippocampal Neurons. *PLOS ONE* 8(4), e58996. doi: 10.1371/journal.pone.0058996.

tom Dieck, S., Kochen, L., Hanus, C., Heumüller, M., Bartnik, I., Nassim-Assir, B., et al. (2015). Direct visualization of newly synthesized target proteins in situ. *Nature Methods* 12(5), 411-414. doi: 10.1038/nmeth.3319.

Tominaga-Yoshino, K., Urakubo, T., Okada, M., Matsuda, H., and Ogura, A. (2008). Repetitive induction of late-phase LTP produces long-lasting synaptic enhancement accompanied by synaptogenesis in cultured hippocampal slices. *Hippocampus* 18(3), 281-293. doi: 10.1002/hipo.20391.

Tominaga-Yoshino, K., Urakubo, T., Ueno, Y., Kawaai, K., Saito, S., Tashiro, T., et al. (2020). Transient appearance of Ca^{2+}-permeable AMPA receptors is crucial for the production of repetitive LTP-induced synaptic enhancement (RISE) in cultured hippocampal slices. *Hippocampus* 30(7), 763-769. doi: 10.1002/hipo.23206.

Tomita, S., Stein, V., Stocker, T.J., Nicoll, R.A., and Bredt, D.S. (2005). Bidirectional Synaptic Plasticity Regulated by Phosphorylation of Stargazin-like TARPs. *Neuron* 45(2), 269-277. doi: https://doi.org/10.1016/j.neuron.2005.01.009.

Torquatto, K.I., Menegolla, A.P., Popik, B., Casagrande, M.A., and de Oliveira Alvares, L. (2019). Role of calcium-permeable AMPA receptors in memory consolidation, retrieval and updating. *Neuropharmacology* 144, 312-318. doi: https://doi.org/10.1016/j.neuropharm.2018.10.030.

Torre, E.R., and Steward, O. (1992). Demonstration of local protein synthesis within dendrites using a new cell culture system that permits the isolation of living axons and dendrites from their cell bodies. *J Neurosci* 12(3), 762-772.

Torre, E.R., and Steward, O. (1996). Protein synthesis within dendrites: glycosylation of newly synthesized proteins in dendrites of hippocampal neurons in culture. *J Neurosci* 16(19), 5967-5978. doi: 10.1523/jneurosci.16-19-05967.1996.

Traynelis, S.F., Wollmuth, L.P., McBain, C.J., Menniti, F.S., Vance, K.M., Ogden, K.K., et al. (2010). Glutamate receptor ion channels: structure, regulation, and function. *Pharmacol Rev* 62(3), 405-496. doi: 10.1124/pr.109.002451.

Trifilieff, P., Rives, M.-L., Urizar, E., Piskorowski, R.A., Vishwasrao, H.D., Castrillon, J., et al. (2011). Detection of antigen interactions ex vivo by proximity ligation assay: endogenous dopamine D2-adenosine A2A receptor complexes in the striatum. *BioTechniques* 51(2), 111-118. doi: 10.2144/000113719.

Tsien, J.Z., Huerta, P.T., and Tonegawa, S. (1996). The Essential Role of Hippocampal CA1 NMDA Receptor–Dependent Synaptic Plasticity in Spatial Memory. *Cell* 87(7), 1327-1338. doi: https://doi.org/10.1016/S0092-8674(00)81827-9.

Tsui, C.C., Copeland, N.G., Gilbert, D.J., Jenkins, N.A., Barnes, C., and Worley, P.F. (1996). Narp, a novel member of the pentraxin family, promotes neurite outgrowth and is dynamically regulated by neuronal activity. *The Journal of Neuroscience* 16(8), 2463-2478.

Tsui, J., and Malenka, R.C. (2006). Substrate Localization Creates Specificity in Calcium/Calmodulin-dependent Protein Kinase II Signaling at Synapses. *Journal of Biological Chemistry* 281(19), 13794-13804. doi: 10.1074/jbc.M600966200.

Tsumoto, T. (1993). Long-term depression in cerebral cortex: a possible substrate of "forgetting" that should not be forgotten. *Neurosci Res* 16(4), 263-270.

Tsuzuki, K., Lambolez, B., Rossier, J., and Ozawa, S. (2001). Absolute quantification of AMPA receptor subunit mRNAs in single hippocampal neurons. *Journal of Neurochemistry* 77(6), 1650-1659. doi: 10.1046/j.1471-4159.2001.00388.x.

Tsvetkov, E., Carlezon, W.A., Benes, F.M., Kandel, E.R., and Bolshakov, V.Y. (2002). Fear conditioning occludes LTP-induced presynaptic enhancement of synaptic transmission in the cortical pathway to the lateral amygdala. *Neuron* 34(2), 289-300. doi: 10.1016/s0896-6273(02)00645-1.

Tukey, D.S., and Ziff, E.B. (2013). Ca^{2+}-permeable AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors and dopamine D1 receptors regulate GluA1 trafficking in striatal neurons. *The Journal of biological chemistry* 288(49), 35297-35306. doi: 10.1074/jbc.M113.516690.

Turner, P.R., Bourne, K., Garama, D., Carne, A., Abraham, W.C., and Tate, W.P. (2007). Production, purification and functional validation of human secreted amyloid precursor proteins for use as neuropharmacological reagents. *Journal of Neuroscience Methods* 164(1), 68-74. doi: https://doi.org/10.1016/j.jneumeth.2007.04.001.

Turrigiano, G. (2012). Homeostatic Synaptic Plasticity: Local and Global Mechanisms for Stabilizing Neuronal Function. *Cold Spring Harb Perspect Biol* 4(1). doi: 10.1101/cshperspect.a005736.

Turrigiano, G.G. (2008). The self-tuning neuron: synaptic scaling of excitatory synapses. *Cell* 135(3), 422-435. doi: 10.1016/j.cell.2008.10.008.

Turrigiano, G.G., and Nelson, S.B. (2004). Homeostatic plasticity in the developing nervous system. *Nat Rev Neurosci* 5(2), 97-107. doi: 10.1038/nrn1327.

Turrigiano, G.G., Leslie, K.R., Desai, N.S., Rutherford, L.C., and Nelson, S.B. (1998). Activity-dependent scaling of quantal amplitude in neocortical neurons. *Nature* 391(6670), 892-896. doi: 10.1038/36103.

Tuszynski, M.H., Thal, L., Pay, M., Salmon, D.P., U, H.S., Bakay, R., et al. (2005). A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. *Nature Medicine* 11(5), 551-555. doi: 10.1038/nm1239.

Twomey, E.C., Yelshanskaya, M.V., Vassilevski, A.A., and Sobolevsky, A.I. (2018). Mechanisms of Channel Block in Calcium-Permeable AMPA Receptors. *Neuron* 99(5), 956-968.e954. doi: https://doi.org/10.1016/j.neuron.2018.07.027.

Tyan, S.-H., Shih, A.Y.-J., Walsh, J.J., Maruyama, H., Sarsoza, F., Ku, L., et al. (2012). Amyloid precursor protein (APP) regulates synaptic structure and function. *Molecular and cellular neurosciences* 51(1-2), 43-52. doi: 10.1016/j.mcn.2012.07.009.

Tyan, S.H., Shih, A.Y., Walsh, J.J., Maruyama, H., Sarsoza, F., Ku, L., et al. (2012). Amyloid precursor protein (APP) regulates synaptic structure and function. *Mol Cell Neurosci* 51(1-2), 43-52. doi: 10.1016/j.mcn.2012.07.009.

Tyler, W.J., and Pozzo-Miller, L.D. (2001). BDNF enhances quantal neurotransmitter release and increases the number of docked vesicles at the active zones of hippocampal excitatory synapses. *The Journal of neuroscience : the official journal of the Society for Neuroscience* 21(12), 4249-4258. doi: 10.1523/JNEUROSCI.21-12-04249.2001.

Ullian, E.M., Sapperstein, S.K., Christopherson, K.S., and Barres, B.A. (2001). Control of synapse number by glia. *Science* 291(5504), 657-661. doi: 10.1126/science.291.5504.657.

Urakubo, T., Ogura, A., and Tominaga-Yoshino, K. (2006). Ultrastructural features of hippocampal CA1 synapses with respect to synaptic enhancement following repeated PKA activation. *Neurosci Lett* 407(1), 1-5. doi: 10.1016/j.neulet.2006.07.057.

Urban, K.R., and Gao, W.-J. (2014). Performance enhancement at the cost of potential brain plasticity: neural ramifications of nootropic drugs in the healthy developing brain. *Frontiers in systems neuroscience* 8, 38-38. doi: 10.3389/fnsys.2014.00038.

Valera, E., Sánchez-Martín, F.J., Ferrer-Montiel, A.V., Messeguer, A., and Merino, J.M. (2008). NMDA-induced neuroprotection in hippocampal neurons is mediated through the protein kinase A and CREB (cAMP-response element-binding protein) pathway. *Neurochem Int* 53(5), 148-154. doi: 10.1016/j.neuint.2008.07.007.

van den Hooff, P., Urban, I.J., and de Wied, D. (1989). Vasopressin maintains long-term potentiation in rat lateral septum slices. *Brain Res* 505(2), 181-186. doi: 10.1016/0006-8993(89)91440-6.

van Dyck, C.H. (2018). Anti-Amyloid-β Monoclonal Antibodies for Alzheimer's Disease: Pitfalls and Promise. *Biological psychiatry* 83(4), 311-319. doi: 10.1016/j.biopsych.2017.08.010.

Van Harreveld, A., and Fifkova, E. (1975). Swelling of dendritic spines in the fascia dentata after stimulation of the perforant fibers as a mechanism of post-tetanic potentiation. *Experimental Neurology* 49(3), 736-749. doi: https://doi.org/10.1016/0014-4886(75)90055-2.

Van Nostrand, W.E., Wagner, S.L., Shankle, W.R., Farrow, J.S., Dick, M., Rozemuller, J.M., et al. (1992). Decreased levels of soluble amyloid beta-protein precursor in cerebrospinal fluid of live Alzheimer disease patients. *Proc Natl Acad Sci U S A* 89(7), 2551-2555.

van Spronsen, M., van Battum, E.Y., Kuijpers, M., Vangoor, V.R., Rietman, M.L., Pothof, J., et al. (2013a). Developmental and activity-dependent miRNA expression profiling in primary hippocampal neuron cultures. *PloS one* 8(10), e74907-e74907. doi: 10.1371/journal.pone.0074907.

VanDongen, A.M. (2008). *Biology of the NMDA Receptor.* CRC Press.

Vann, S.D., Brown, M.W., Erichsen, J.T., and Aggleton, J.P. (2000). Fos imaging reveals differential patterns of hippocampal and parahippocampal subfield activation in rats in response to different spatial memory tests. *J Neurosci* 20(7), 2711-2718.

Vazdarjanova, A., McNaughton, B.L., Barnes, C.A., Worley, P.F., and Guzowski, J.F. (2002). Experience-dependent coincident expression of the effector immediate-early genes arc and Homer 1a in hippocampal and neocortical neuronal networks. *J Neurosci* 22(23), 10067-10071. doi: 10.1523/jneurosci.22-23-10067.2002.

Verdoorn, T., Burnashev, N., Monyer, H., Seeburg, P., and Sakmann, B. (1991). Structural determinants of ion flow through recombinant glutamate receptor channels. *Science* 252(5013), 1715-1718. doi: 10.1126/science.1710829.

Verghese, P.B., Castellano, J.M., Garai, K., Wang, Y., Jiang, H., Shah, A., et al. (2013). ApoE influences amyloid-β (Aβ) clearance despite minimal apoE/Aβ association in physiological conditions. *Proc Natl Acad Sci U S A* 110(19), E1807-1816. doi: 10.1073/pnas.1220484110.

Verkhratsky, A., and Kirchhoff, F. (2007). Glutamate-mediated neuronal–glial transmission. *Journal of Anatomy* 210(6), 651-660. doi: 10.1111/j.1469-7580.2007.00734.x.

Verstraelen, P., Van Dyck, M., Verschuuren, M., Kashikar, N.D., Nuydens, R., Timmermans, J.-P., et al. (2018). Image-Based Profiling of Synaptic Connectivity in Primary Neuronal Cell Culture. *Frontiers in Neuroscience* 12(389). doi: 10.3389/fnins.2018.00389.

Vickers, C.A., and Wyllie, D.J.A. (2007). Late-phase, protein synthesis-dependent long-term potentiation in hippocampal CA1 pyramidal neurones with destabilized microtubule networks. *British journal of pharmacology* 151(7), 1071-1077. doi: 10.1038/sj.bjp.0707314.

Vickers, C.A., Dickson, K.S., and Wyllie, D.J.A. (2005). Induction and maintenance of late-phase long-term potentiation in isolated dendrites of rat hippocampal CA1 pyramidal neurones. *The Journal of Physiology* 568(Pt 3), 803-813. doi: 10.1113/jphysiol.2005.092924.

Villani, F., and Johnston, D. (1993). Serotonin inhibits induction of long-term potentiation at commissural synapses in hippocampus. *Brain Res* 606(2), 304-308. doi: 10.1016/0006-8993(93)90998-3.

Villarreal, D.M., Do, V., Haddad, E., and Derrick, B.E. (2002). NMDA receptor antagonists sustain LTP and spatial memory: active processes mediate LTP decay. *Nat Neurosci* 5(1), 48-52. doi: 10.1038/nn776.

Vincent-Lamarre, P., Lynn, M., and Béïque, J.-C. (2018). The Eloquent Silent Synapse. *Trends in Neurosciences* 41(9), 557-559. doi: https://doi.org/10.1016/j.tins.2018.07.002.

Vogt, K., Lüscher, H.R., and Streit, J. (1995). Analysis of synaptic transmission at single identified boutons on rat spinal neurons in culture. *Pflügers Archiv* 430(6), 1022-1028. doi: 10.1007/BF01837420.

von Bartheld, C.S., Kinoshita, Y., Prevette, D., Yin, Q.W., Oppenheim, R.W., and Bothwell, M. (1994). Positive and negative effects of neurotrophins on the isthmo-optic nucleus in chick embryos. *Neuron* 12(3), 639-654. doi: 10.1016/0896-6273(94)90219-4.

von Koch, C.S., Zheng, H., Chen, H., Trumbauer, M., Thinakaran, G., van der Ploeg, L.H., et al. (1997). Generation of APLP2 KO mice and early postnatal lethality in APLP2/APP double KO mice. *Neurobiol Aging* 18(6), 661-669. doi: 10.1016/s0197-4580(97)00151-6.

Waites, C.L., Specht, C.G., Härtel, K., Leal-Ortiz, S., Genoux, D., Li, D., et al. (2009). Synaptic SAP97 Isoforms Regulate AMPA Receptor Dynamics and Access to Presynaptic Glutamate. *J Neurosci* 29(14), 4332-4345. doi: 10.1523/jneurosci.4431-08.2009.

Walikonis, R.S., Oguni, A., Khorosheva, E.M., Jeng, C.-J., Asuncion, F.J., and Kennedy, M.B. (2001). Densin-180 Forms a Ternary Complex with the α-Subunit of Ca²⁺/Calmodulin-Dependent Protein Kinase II and α-Actinin. *The Journal of Neuroscience* 21(2), 423-433. doi: 10.1523/jneurosci.21-02-00423.2001.

Wall, M.J., and Corrêa, S.A.L. (2018). The mechanistic link between Arc/Arg3.1 expression and AMPA receptor endocytosis. *Seminars in Cell & Developmental Biology* 77, 17-24. doi: https://doi.org/10.1016/j.semcdb.2017.09.005.

Wall, M.J., Collins, D.R., Chery, S.L., Allen, Z.D., Pastuzyn, E.D., George, A.J., et al. (2018). The Temporal Dynamics of Arc Expression Regulate Cognitive Flexibility. *Neuron* 98(6), 1124-1132.e1127. doi: 10.1016/j.neuron.2018.05.012.

Wallace, C.S., Lyford, G.L., Worley, P.F., and Steward, O. (1998). Differential Intracellular Sorting of Immediate Early Gene mRNAs Depends on Signals in the mRNA Sequence. *The Journal of Neuroscience* 18(1), 26-35.

Wallace, T.L., and Johnson, E.M., Jr. (1989). Cytosine arabinoside kills postmitotic neurons: evidence that deoxycytidine may have a role in neuronal survival that is independent of DNA synthesis. *J Neurosci* 9(1), 115-124. doi: 10.1523/jneurosci.09-01-00115.1989.

Walsh, D.A., Perkins, J.P., and Krebs, E.G. (1968). An Adenosine 3',5'-Monophosphate-dependant Protein Kinase from Rabbit Skeletal Muscle. *Journal of Biological Chemistry* 243(13), 3763-3765.

Waltereit, R., Dammermann, B., Wulff, P., Scafidi, J., Staubli, U., Kauselmann, G., et al. (2001). Arg3.1/Arc mRNA induction by Ca^{2+} and cAMP requires protein kinase A and mitogen-activated protein kinase/extracellular regulated kinase activation. *J Neurosci* 21(15), 5484-5493. doi: 10.1523/jneurosci.21-15-05484.2001.

Walton, S.P., Wu, M., Gredell, J.A., and Chan, C. (2010). Designing highly active siRNAs for therapeutic applications. *Febs j* 277(23), 4806-4813. doi: 10.1111/j.1742-4658.2010.07903.x.

Wang, H.-G., Lu, F.-M., Jin, I., Udo, H., Kandel, E.R., de Vente, J., et al. (2005). Presynaptic and Postsynaptic Roles of NO, cGK, and RhoA in Long-Lasting Potentiation and Aggregation of Synaptic Proteins. *Neuron* 45(3), 389-403. doi: https://doi.org/10.1016/j.neuron.2005.01.011.

Wang, H.-Y., Lee, D.H.S., Davis, C.B., and Shank, R.P. (2000a). Amyloid Peptide Aβ1-42 Binds Selectively and with Picomolar Affinity to α7 Nicotinic Acetylcholine Receptors. *Journal of Neurochemistry* 75(3), 1155-1161. doi: 10.1046/j.1471-4159.2000.0751155.x.

Wang, H., Megill, A., He, K., Kirkwood, A., and Lee, H.-K. (2012). Consequences of Inhibiting Amyloid Precursor Protein Processing Enzymes on Synaptic Function and Plasticity. *Neural Plasticity* 2012, 272374. doi: 10.1155/2012/272374.

Wang, H.L., Tsai, L.Y., and Lee, E.H. (2000b). Corticotropin-releasing factor produces a protein synthesis--dependent long-lasting potentiation in dentate gyrus neurons. *J Neurophysiol* 83(1), 343-349. doi: 10.1152/jn.2000.83.1.343.

Wang, L.Y., Salter, M.W., and MacDonald, J.F. (1991). Regulation of kainate receptors by cAMP-dependent protein kinase and phosphatases. *Science* 253(5024), 1132-1135.

Wang, M., Jing, T., Wang, X., and Yao, D. (2018). Beta-secretase/BACE1 promotes APP endocytosis and processing in the endosomes and on cell membrane. *Neurosci Lett* 685, 63-67. doi: 10.1016/j.neulet.2018.08.016.

Wang, X., and Proud, C.G. (2006). The mTOR pathway in the control of protein synthesis. *Physiology (Bethesda)* 21, 362-369. doi: 10.1152/physiol.00024.2006.

Wang, Y., Dong, Q., Xu, X.-F., Feng, X., Xin, J., Wang, D.-D., et al. (2013). Phosphorylation of Cofilin Regulates Extinction of Conditioned Aversive Memory via AMPAR Trafficking. *The Journal of Neuroscience* 33(15), 6423-6433. doi: 10.1523/jneurosci.5107-12.2013.

Wang, Z., Edwards, J.G., Riley, N., Provance, D.W., Jr., Karcher, R., Li, X.-D., et al. (2008). Myosin Vb mobilizes recycling endosomes and AMPA receptors for postsynaptic plasticity. *Cell* 135(3), 535-548. doi: 10.1016/j.cell.2008.09.057.

Wang, Z., Wang, B., Yang, L., Guo, Q., Aithmitti, N., Songyang, Z., et al. (2009). Presynaptic and postsynaptic interaction of the amyloid precursor protein promotes peripheral and central synaptogenesis. *J Neurosci* 29(35), 10788-10801. doi: 10.1523/jneurosci.2132-09.2009.

Wasling, P., Strandberg, J., and Hanse, E. (2012). AMPA Receptor Activation Causes Silencing of AMPA Receptor-Mediated Synaptic Transmission in the Developing Hippocampus. *PLOS ONE* 7(4), e34474. doi: 10.1371/journal.pone.0034474.

Watt, A.J., Sjostrom, P.J., Hausser, M., Nelson, S.B., and Turrigiano, G.G. (2004). A proportional but slower NMDA potentiation follows AMPA potentiation in LTP. *Nat Neurosci* 7(5), 518-524. doi: 10.1038/nn1220.

Waung, M.W., Pfeiffer, B.E., Nosyreva, E.D., Ronesi, J.A., and Huber, K.M. (2008). Rapid Translation of Arc/Arg3.1 Selectively Mediates mGluR-Dependent LTD through Persistent Increases in AMPAR Endocytosis Rate. *Neuron* 59(1), 84-97. doi: https://doi.org/10.1016/j.neuron.2008.05.014.

Wee, C.L., Teo, S., Oey, N.E., Wright, G.D., VanDongen, H.M.A., and VanDongen, A.M.J. (2014). Nuclear Arc Interacts with the Histone Acetyltransferase Tip60 to Modify H4K12 Acetylation(1,2,3). *eNeuro* 1(1), ENEURO.0019-0014.2014. doi: 10.1523/ENEURO.0019-14.2014.

Wen, J.A., and Barth, A.L. (2012). Synaptic lability after experience-dependent plasticity is not mediated by calcium-permeable AMPARs. *Frontiers in molecular neuroscience* 5, 15-15. doi: 10.3389/fnmol.2012.00015.

Wenthold, R., Petralia, R., Blahos J, I., and Niedzielski, A. (1996a). Evidence for multiple AMPA receptor complexes in hippocampal CA1/CA2 neurons. *The Journal of Neuroscience* 16(6), 1982-1989. doi: 10.1523/jneurosci.16-06-01982.1996.

Werner, C.T., Murray, C.H., Reimers, J.M., Chauhan, N.M., Woo, K.K., Molla, H.M., et al. (2017). Trafficking of calcium-permeable and calcium-impermeable AMPA receptors in nucleus accumbens medium spiny neurons co-cultured with prefrontal cortex neurons. *Neuropharmacology* 116, 224-232. doi: 10.1016/j.neuropharm.2016.12.014.

Westphal, R.S., Tavalin, S.J., Lin, J.W., Alto, N.M., Fraser, I.D., Langeberg, L.K., et al. (1999). Regulation of NMDA receptors by an associated phosphatase-kinase signaling complex. *Science* 285(5424), 93-96.

Weyer, S.W., Klevanski, M., Delekate, A., Voikar, V., Aydin, D., Hick, M., et al. (2011). APP and APLP2 are essential at PNS and CNS synapses for transmission, spatial learning and LTP. *Embo j* 30(11), 2266-2280. doi: 10.1038/emboj.2011.119.

Weyer, S.W., Zagrebelsky, M., Herrmann, U., Hick, M., Ganss, L., Gobbert, J., et al. (2014). Comparative analysis of single and combined APP/APLP knockouts reveals reduced spine density in APP-KO mice that is prevented by APPsα expression. *Acta Neuropathologica Communications* 2(1), 36. doi: 10.1186/2051-5960-2-36.

Wezenberg, E., Jan Verkes, R., Ruigt, G.S.F., Hulstijn, W., and Sabbe, B.G.C. (2007). Acute Effects of the Ampakine Farampator on Memory and Information Processing in Healthy Elderly Volunteers. *Neuropsychopharmacology* 32(6), 1272-1283. doi: 10.1038/sj.npp.1301257.

Wheeler, D.G., Barrett, C.F., Groth, R.D., Safa, P., and Tsien, R.W. (2008). CaMKII locally encodes L-type channel activity to signal to nuclear CREB in excitation-transcription coupling. *J Cell Biol* 183(5), 849-863. doi: 10.1083/jcb.200805048.

Wheeler, D.G., Groth, R.D., Ma, H., Barrett, C.F., Owen, S.F., Safa, P., et al. (2012). Ca(V)1 and Ca(V)2 channels engage distinct modes of Ca(2+) signaling to control CREB-dependent gene expression. *Cell* 149(5), 1112-1124. doi: 10.1016/j.cell.2012.03.041.

White, A.R., Reyes, R., Mercer, J.F., Camakaris, J., Zheng, H., Bush, A.I., et al. (1999). Copper levels are increased in the cerebral cortex and liver of APP and APLP2 knockout mice. *Brain Res* 842(2), 439-444. doi: 10.1016/s0006-8993(99)01861-2.

Whitehead, G., Regan, P., Whitcomb, D.J., and Cho, K. (2017). Ca^{2+}-permeable AMPA receptor: A new perspective on amyloid-beta mediated pathophysiology of Alzheimer's disease. *Neuropharmacology* 112, 221-227. doi: https://doi.org/10.1016/j.neuropharm.2016.08.022.

Whitlock, J.R., Heynen, A.J., Shuler, M.G., and Bear, M.F. (2006a). Learning Induces Long-Term Potentiation in the Hippocampus. *Science* 313(5790), 1093-1097. doi: 10.1126/science.1128134.

Whitlock, J.R., Heynen, A.J., Shuler, M.G., and Bear, M.F. (2006b). Learning induces long-term potentiation in the hippocampus. *Science* 313(5790), 1093-1097. doi: 10.1126/science.1128134.

Whitt, J.L., Petrus, E., and Lee, H.-K. (2014). Experience-dependent homeostatic synaptic plasticity in neocortex. *Neuropharmacology* 78, 45-54. doi: 10.1016/j.neuropharm.2013.02.016.

Whittaker, E., Vereker, E., and Lynch, M.A. (1999). Neuropeptide Y inhibits glutamate release and long-term potentiation in rat dentate gyrus. *Brain Res* 827(1-2), 229-233. doi: 10.1016/s0006-8993(99)01302-5.

Whitwell, J.L. (2010). Progression of atrophy in Alzheimer's disease and related disorders. *Neurotox Res* 18(3-4), 339-346. doi: 10.1007/s12640-010-9175-1.

Wible, C.G. (2013). Hippocampal Physiology, Structure and Function and the Neuroscience of Schizophrenia: A Unified Account of Declarative Memory Deficits, Working Memory Deficits and Schizophrenic Symptoms. *Behav Sci (Basel)* 3(2), 298-315. doi: 10.3390/bs3020298.

Wiegert, J.S., and Oertner, T.G. (2013). Long-term depression triggers the selective elimination of weakly integrated synapses. *Proc Natl Acad Sci U S A* 110(47), E4510-4519. doi: 10.1073/pnas.1315926110.

Wierenga, C.J., Ibata, K., and Turrigiano, G.G. (2005). Postsynaptic expression of homeostatic plasticity at neocortical synapses. *J Neurosci* 25(11), 2895-2905. doi: 10.1523/jneurosci.5217-04.2005.

Wigstrom, H., and Gustafsson, B. (1985a). Facilitation of hippocampal long-lasting potentiation by GABA antagonists. *Acta Physiol Scand* 125(1), 159-172. doi: 10.1111/j.1748-1716.1985.tb07703.x.

Wigstrom, H., and Gustafsson, B. (1985b). On long-lasting potentiation in the hippocampus: a proposed mechanism for its dependence on coincident pre- and postsynaptic activity. *Acta Physiol Scand* 123(4), 519-522. doi: 10.1111/j.1748-1716.1985.tb07621.x.

Wikström, M.A., Matthews, P., Roberts, D., Collingridge, G.L., and Bortolotto, Z.A. (2003). Parallel kinase cascades are involved in the induction of LTP at hippocampal CA1 synapses. *Neuropharmacology* 45(6), 828-836. doi: 10.1016/s0028-3908(03)00336-8.

Willem, M., Tahirovic, S., Busche, M.A., Ovsepian, S.V., Chafai, M., Kootar, S., et al. (2015). η-Secretase processing of APP inhibits neuronal activity in the hippocampus. *Nature* 526(7573), 443-447. doi: 10.1038/nature14864.

Williams, J.M., Guevremont, D., Mason-Parker, S.E., Luxmanan, C., Tate, W.P., and Abraham, W.C. (2007). Differential trafficking of AMPA and NMDA receptors during long-term potentiation in awake adult animals. *J Neurosci* 27(51), 14171-14178. doi: 10.1523/jneurosci.2348-07.2007.

Williams, L.R. (1991). Hypophagia is induced by intracerebroventricular administration of nerve growth factor. *Exp Neurol* 113(1), 31-37. doi: 10.1016/0014-4886(91)90143-z.

Williams, S.R., Wozny, C., and Mitchell, S.J. (2007d). The Back and Forth of Dendritic Plasticity. *Neuron* 56(6), 947-953. doi: 10.1016/j.neuron.2007.12.004.

Willis, B., Martenyi, F., Dean, R., Lowe, S., Nakano, M., Monk, S., et al. (2012). P3-359: Central BACE1 inhibition by LY2886721 produces opposing effects on APP processing as reflected by cerebrospinal fluid sAPPalpha and sAPPbeta. *Alzheimer's & Dementia* 8(4S_Part_16), P582-P582. doi: 10.1016/j.jalz.2012.05.1584.

Wiltgen, B.J., Royle, G.A., Gray, E.E., Abdipranoto, A., Thangthaeng, N., Jacobs, N., et al. (2010). A Role for Calcium-Permeable AMPA Receptors in Synaptic Plasticity and Learning. *PLOS ONE* 5(9), e12818. doi: 10.1371/journal.pone.0012818.

Witter, M.P., and Groenewegen, H.J. (1990). The subiculum: cytoarchitectonically a simple structure, but hodologically complex. *Prog Brain Res* 83, 47-58. doi: 10.1016/s0079-6123(08)61240-6.

Wolfe, M.S. (2007). When loss is gain: reduced presenilin proteolytic function leads to increased Abeta42/Abeta40. Talking Point on the role of presenilin mutations in Alzheimer disease. *EMBO Rep* 8(2), 136-140. doi: 10.1038/sj.embor.7400896.

Woo, N.H., Teng, H.K., Siao, C.J., Chiaruttini, C., Pang, P.T., Milner, T.A., et al. (2005). Activation of p75NTR by proBDNF facilitates hippocampal long-term depression. *Nat Neurosci* 8(8), 1069-1077. doi: 10.1038/nn1510.

Wosiski-Kuhn, M., and Stranahan, A.M. (2012). Transient increases in dendritic spine density contribute to dentate gyrus long-term potentiation. *Synapse (New York, N.Y.)* 66(7), 661-664. doi: 10.1002/syn.21545.

Wozniak, D.F., Xiao, M., Xu, L., Yamada, K.A., and Ornitz, D.M. (2007). Impaired spatial learning and defective theta burst induced LTP in mice lacking fibroblast growth factor 14. *Neurobiol Dis* 26(1), 14-26. doi: 10.1016/j.nbd.2006.11.014.

Wright, A., and Vissel, B. (2012). The essential role of AMPA receptor GluR2 subunit RNA editing in the normal and diseased brain. *Front Mol Neurosci* 5. doi: 10.3389/fnmol.2012.00034.

Wright, B.J., and Jackson, M.B. (2014). Long-Term Potentiation in Hilar Circuitry Modulates Gating by the Dentate Gyrus. *The Journal of Neuroscience* 34(29), 9743-9753. doi: 10.1523/jneurosci.0814-14.2014.

Wright, W.J., Graziane, N.M., Neumann, P.A., Hamilton, P.J., Cates, H.M., Fuerst, L., et al. (2020). Silent synapses dictate cocaine memory destabilization and reconsolidation. *Nature Neuroscience* 23(1), 32-46. doi: 10.1038/s41593-019-0537-6.

Wu, D. (2005). Neuroprotection in experimental stroke with targeted neurotrophins. *NeuroRx : the journal of the American Society for Experimental NeuroTherapeutics* 2(1), 120-128. doi: 10.1602/neurorx.2.1.120.

Wu, H., Nash, J.E., Zamorano, P., and Garner, C.C. (2002). Interaction of SAP97 with minus-end-directed actin motor myosin VI. Implications for AMPA receptor trafficking. *J Biol Chem* 277(34), 30928-30934. doi: 10.1074/jbc.M203735200.

Wu, J., Khan, G.M., and Nichols, R.A. (2007). Dopamine release in prefrontal cortex in response to beta-amyloid activation of alpha7 * nicotinic receptors. *Brain Res* 1182, 82-89. doi: 10.1016/j.brainres.2007.08.079.

Wu, J., Petralia, R.S., Kurushima, H., Patel, H., Jung, M.Y., Volk, L., et al. (2011). Arc/Arg3.1 regulates an endosomal pathway essential for activity-dependent beta-amyloid generation. *Cell* 147(3), 615-628. doi: 10.1016/j.cell.2011.09.036.

Wu, J., Rowan, M.J., and Anwyl, R. (2006). Long-term potentiation is mediated by multiple kinase cascades involving CaMKII or either PKA or p42/44 MAPK in the adult rat dentate gyrus in vitro. *J Neurophysiol* 95(6), 3519-3527. doi: 10.1152/jn.01235.2005.

Wu, L., and Saggau, P. (1994). Presynaptic calcium is increased during normal synaptic transmission and paired-pulse facilitation, but not in long-term potentiation in area CA1 of hippocampus. *The Journal of Neuroscience* 14(2), 645-654.

Wu, X., Zheng, W.J., Lv, M.H., Su, S.H., Zhang, S.J., and Gao, J.F. (2018a). [Effect of different concentrations of calcitonin gene-related peptide on the long-term potentiation in hippocampus of mice]. *Sheng Li Xue Bao* 70(1), 17-22.

Wu, Y., Yuan, M., Su, W., Zhu, M., Yao, X., Wang, Y., et al. (2018b). The constitutively active PKG II mutant effectively inhibits gastric cancer development via a blockade of EGF/EGFR-associated signalling cascades. *Ther Adv Med Oncol* 10, 1758834017751635. doi: 10.1177/1758834017751635.

Wu, Y.K., Fujishima, K., and Kengaku, M. (2015). Differentiation of Apical and Basal Dendrites in Pyramidal Cells and Granule Cells in Dissociated Hippocampal Cultures. *PLOS ONE* 10(2), e0118482. doi: 10.1371/journal.pone.0118482.

Xia, J., Chung, H.J., Wihler, C., Huganir, R.L., and Linden, D.J. (2000). Cerebellar Long-Term Depression Requires PKC-Regulated Interactions between GluR2/3 and PDZ Domain–Containing Proteins. *Neuron* 28(2), 499-510. doi: https://doi.org/10.1016/S0896-6273(00)00128-8.

Xia, J., Meyers, A.M., and Beeler, J.A. (2017). Chronic Nicotine Alters Corticostriatal Plasticity in the Striatopallidal Pathway Mediated By NR2B-Containing Silent Synapses. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* 42(12), 2314-2324. doi: 10.1038/npp.2017.87.

Xia, J., Zhang, X., Staudinger, J., and Huganir, R.L. (1999). Clustering of AMPA Receptors by the Synaptic PDZ Domain–Containing Protein PICK1. *Neuron* 22(1), 179-187. doi: https://doi.org/10.1016/S0896-6273(00)80689-3.

Xia, Z., Dudek, H., Miranti, C.K., and Greenberg, M.E. (1996a). Calcium Influx via the NMDA Receptor Induces Immediate Early Gene Transcription by a MAP Kinase/ERK-Dependent Mechanism. *The Journal of Neuroscience* 16(17), 5425-5436. doi: 10.1523/jneurosci.16-17-05425.1996.

Xia, Z., Dudek, H., Miranti, C.K., and Greenberg, M.E. (1996b). Calcium influx via the NMDA receptor induces immediate early gene transcription by a MAP kinase/ERK-dependent mechanism. *Journal of Neuroscience* 16(17), 5425-5436.

Xiao, M.-Y., Wasling, P., Hanse, E., and Gustafsson, B. (2004). Creation of AMPA-silent synapses in the neonatal hippocampus. *Nature Neuroscience* 7(3), 236-243. doi: 10.1038/nn1196.

Xie, H., Liu, Y., Zhu, Y., Ding, X., Yang, Y., and Guan, J.S. (2014). In vivo imaging of immediate early gene expression reveals layer-specific memory traces in the mammalian brain. *Proc Natl Acad Sci U S A* 111(7), 2788-2793. doi: 10.1073/pnas.1316808111.

Xie, X., Berger, T.W., and Barrionuevo, G. (1992). Isolated NMDA receptor-mediated synaptic responses express both LTP and LTD. *J Neurophysiol* 67(4), 1009-1013. doi: 10.1152/jn.1992.67.4.1009.

Xiong, H., Boyle, J., Winkelbauer, M., Gorantla, S., Zheng, J., Ghorpade, A., et al. (2003). Inhibition of long-term potentiation by interleukin-8: implications for human immunodeficiency virus-1-associated dementia. *J Neurosci Res* 71(4), 600-607. doi: 10.1002/jnr.10503.

Xiong, H., McCabe, L., Costello, J., Anderson, E., Weber, G., and Ikezu, T. (2004). Activation of NR1a/NR2B receptors by soluble factors from APP-stimulated monocyte-derived macrophages: implications for the pathogenesis of Alzheimer's disease. *Neurobiology of Aging* 25(7), 905-911. doi: https://doi.org/10.1016/j.neurobiolaging.2003.09.007.

Xiong, M., Jones, O.D., Peppercorn, K., Ohline, S.M., Tate, W.P., and Abraham, W.C. (2017). Secreted amyloid precursor protein-alpha can restore novel object location memory and hippocampal LTP in aged rats. *Neurobiol Learn Mem* 138, 291-299. doi: 10.1016/j.nlm.2016.08.002.

Xu, B., Michalski, B., Racine, R.J., and Fahnestock, M. (2004). The effects of brain-derived neurotrophic factor (BDNF) administration on kindling induction, Trk expression and seizure-related

morphological changes. *Neuroscience* 126(3), 521-531. doi: 10.1016/j.neuroscience.2004.03.044.

Xu, C., Liu, H.-J., Qi, L., Tao, C.-L., Wang, Y.-J., Shen, Z., et al. (2020). Structure and plasticity of silent synapses in developing hippocampal neurons visualized by super-resolution imaging. *Cell Discovery* 6(1), 8. doi: 10.1038/s41421-019-0139-1.

Xu, F., Previti, M.L., Nieman, M.T., Davis, J., Schmaier, A.H., and Van Nostrand, W.E. (2009). AbetaPP/APLP2 family of Kunitz serine proteinase inhibitors regulate cerebral thrombosis. *J Neurosci* 29(17), 5666-5670. doi: 10.1523/jneurosci.0095-09.2009.

Xu, J., and Wu, L.G. (2005). The decrease in the presynaptic calcium current is a major cause of short-term depression at a calyx-type synapse. *Neuron* 46(4), 633-645. doi: 10.1016/j.neuron.2005.03.024.

Xu, W. (2011). PSD-95-like membrane associated guanylate kinases (PSD-MAGUKs) and synaptic plasticity. *Current Opinion in Neurobiology* 21(2), 306-312. doi: https://doi.org/10.1016/j.conb.2011.03.001.

Xu, Z., Adler, A., Li, H., Pérez-Cuesta, L.M., Lai, B., Li, W., et al. (2019). Fear conditioning and extinction induce opposing changes in dendritic spine remodeling and somatic activity of layer 5 pyramidal neurons in the mouse motor cortex. *Scientific Reports* 9(1), 4619. doi: 10.1038/s41598-019-40549-y.

Yamagata, K., Sanders, L.K., Kaufmann, W.E., Yee, W., Barnes, C.A., Nathans, D., et al. (1994). rheb, a growth factor- and synaptic activity-regulated gene, encodes a novel Ras-related protein. *J Biol Chem* 269(23), 16333-16339.

Yamasaki, M., Fukaya, M., Yamazaki, M., Azechi, H., Natsume, R., Abe, M., et al. (2016). TARP γ-2 and γ-8 Differentially Control AMPAR Density Across Schaffer Collateral/Commissural Synapses in the Hippocampal CA1 Area. *The Journal of Neuroscience* 36(15), 4296-4312. doi: 10.1523/jneurosci.4178-15.2016.

Yan, X., Liu, J., Ye, Z., Huang, J., He, F., Xiao, W., et al. (2016). CaMKII-Mediated CREB Phosphorylation Is Involved in Ca^{2+}-Induced BDNF mRNA Transcription and Neurite Outgrowth Promoted by Electrical Stimulation. *PloS one* 11(9), e0162784-e0162784. doi: 10.1371/journal.pone.0162784.

Yang, J.H., Seo, S.Y., Oh, J.H., Ryu, I.S., Kim, J., Lee, D.K., et al. (2018). Activation of Protein Kinase G After Repeated Cocaine Administration Is Necessary for the Phosphorylation of α-Amino-3-Hydroxy-5-Methyl-4-Isoxazolepropionic Acid Receptor GluA1 at Serine 831 in the Rat Nucleus Accumbens. *Frontiers in Molecular Neuroscience* 11(263). doi: 10.3389/fnmol.2018.00263.

Yang, W.N., Ma, K.G., Chen, X.L., Shi, L.L., Bu, G., Hu, X.D., et al. (2014). Mitogen-activated protein kinase signaling pathways are involved in regulating α7 nicotinic acetylcholine receptor-mediated amyloid-β uptake in SH-SY5Y cells. *Neuroscience* 278, 276-290. doi: https://doi.org/10.1016/j.neuroscience.2014.08.013.

Yang, Y., Wang, X.-b., and Zhou, Q. (2010). Perisynaptic GluR2-lacking AMPA receptors control the reversibility of synaptic and spines modifications. *Proceedings of the National Academy of Sciences* 107(26), 11999-12004. doi: 10.1073/pnas.0913004107.

Yang, Y., Wang, X.-b., Frerking, M., and Zhou, Q. (2008). Delivery of AMPA receptors to perisynaptic sites precedes the full expression of long-term potentiation. *Proceedings of the National Academy of Sciences* 105(32), 11388-11393. doi: 10.1073/pnas.0802978105.

Yao, Y., Kelly, M.T., Sajikumar, S., Serrano, P., Tian, D., Bergold, P.J., et al. (2008). PKMζ Maintains Late Long-Term Potentiation by N-Ethylmaleimide-Sensitive Factor/GluR2-Dependent Trafficking of Postsynaptic AMPA Receptors. *The Journal of Neuroscience* 28(31), 7820-7827. doi: 10.1523/jneurosci.0223-08.2008.

Yasuda, H., Barth, A.L., Stellwagen, D., and Malenka, R.C. (2003). A developmental switch in the signaling cascades for LTP induction. *Nat Neurosci* 6(1), 15-16. doi: 10.1038/nn985.

Yasui, M., and Kawasaki, K. (1995). CCKB-receptor activation augments the long-term potentiation in guinea pig hippocampal slices. *Jpn J Pharmacol* 68(4), 441-447. doi: 10.1254/jjp.68.441.

Yates, D. (2012). Direct involvement for astrocytes in LTP? *Nature Reviews Neuroscience* 13(4), 221-221. doi: 10.1038/nrn3221.

Yilmaz-Rastoder, E., Miyamae, T., Braun, A.E., and Thiels, E. (2011). LTP- and LTD-inducing stimulations cause opposite changes in arc/arg3.1 mRNA level in hippocampal area CA1 in vivo. *Hippocampus* 21(12), 1290-1301. doi: 10.1002/hipo.20838.

Yin, Y., Edelman, G.M., and Vanderklish, P.W. (2002). The brain-derived neurotrophic factor enhances synthesis of Arc in synaptoneurosomes. *Proc Natl Acad Sci U S A* 99(4), 2368-2373. doi: 10.1073/pnas.042693699.

Ying, S.-W., Futter, M., Rosenblum, K., Webber, M.J., Hunt, S.P., Bliss, T.V.P., et al. (2002). Brain-Derived Neurotrophic Factor Induces Long-Term Potentiation in Intact Adult Hippocampus: Requirement for ERK Activation Coupled to CREB and Upregulation of Arc Synthesis. *The Journal of Neuroscience* 22(5), 1532-1540. doi: 10.1523/jneurosci.22-05-01532.2002.

Yogev, S., and Shen, K. (2017). Establishing Neuronal Polarity with Environmental and Intrinsic Mechanisms. *Neuron* 96(3), 638-650. doi: 10.1016/j.neuron.2017.10.021.

Yoshihara, M., and Montana, E.S. (2004). The Synaptotagmins: Calcium Sensors for Vesicular Trafficking. *The Neuroscientist* 10(6), 566-574. doi: 10.1177/1073858404268770.

Young-Pearse, T.L., Bai, J., Chang, R., Zheng, J.B., LoTurco, J.J., and Selkoe, D.J. (2007). A critical function for beta-amyloid precursor protein in neuronal migration revealed by in utero RNA interference. *J Neurosci* 27(52), 14459-14469. doi: 10.1523/jneurosci.4701-07.2007.

Young-Pearse, T.L., Chen, A.C., Chang, R., Marquez, C., and Selkoe, D.J. (2008). Secreted APP regulates the function of full-length APP in neurite outgrowth through interaction with integrin beta1. *Neural Dev* 3, 15. doi: 10.1186/1749-8104-3-15.

Young, J.Z., Isiegas, C., Abel, T., and Nguyen, P.V. (2006). Metaplasticity of the late-phase of long-term potentiation: a critical role for protein kinase A in synaptic tagging. *The European journal of neuroscience* 23(7), 1784-1794. doi: 10.1111/j.1460-9568.2006.04707.x.

Yu, X.-M., and Salter, M.W. (1998). Gain control of NMDA-receptor currents by intracellular sodium. *Nature* 396, 469. doi: 10.1038/24877

Yudowski, G.A., Puthenveedu, M.A., Leonoudakis, D., Panicker, S., Thorn, K.S., Beattie, E.C., et al. (2007). Real-time imaging of discrete exocytic events mediating surface delivery of AMPA receptors. *J Neurosci* 27(41), 11112-11121. doi: 10.1523/jneurosci.2465-07.2007.

Yusof, H.H., Lee, H.-C., Seth, E.A., Wu, X., Hewitt, C.A., Scott, H.S., et al. (2019). Expression Profiling of Notch Signalling Pathway and Gamma-Secretase Activity in the Brain of Ts1Cje Mouse Model of Down Syndrome. *Journal of Molecular Neuroscience* 67(4), 632-642. doi: 10.1007/s12031-019-01275-2.

Yuste, R. (2015). The discovery of dendritic spines by Cajal. *Frontiers in neuroanatomy* 9, 18-18. doi: 10.3389/fnana.2015.00018.

Zalutsky, R.A., and Nicoll, R.A. (1990). Comparison of two forms of long-term potentiation in single hippocampal neurons. *Science* 248(4963), 1619-1624. doi: 10.1126/science.2114039.

Zamanillo, D., Sprengel, R., Hvalby, O., Jensen, V., Burnashev, N., Rozov, A., et al. (1999). Importance of AMPA receptors for hippocampal synaptic plasticity but not for spatial learning. *Science* 284(5421), 1805-1811.

Zhai, X., Leo, M.D., and Jaggar, J.H. (2017). Endothelin-1 Stimulates Vasoconstriction Through Rab11A Serine 177 Phosphorylation. *Circ Res* 121(6), 650-661. doi: 10.1161/circresaha.117.311102.

Zhang, H., Li, T., Li, S., and Liu, F. (2016a). Cross-talk between α7 nAchR and NMDAR revealed by protein profiling. *J Proteomics* 131, 113-121. doi: 10.1016/j.jprot.2015.10.018.

Zhang, H., Wu, L., Pchitskaya, E., Zakharova, O., Saito, T., Saido, T., et al. (2015). Neuronal Store-Operated Calcium Entry and Mushroom Spine Loss in Amyloid Precursor Protein Knock-In Mouse Model of Alzheimer's Disease. The Journal of neuroscience : the official journal of the Society for Neuroscience 35(39), 13275-13286. doi: 10.1523/JNEUROSCI.1034-15.2015.

Zhang, J., Guo, J., Zhao, X., Chen, Z., Wang, G., Liu, A., et al. (2013). Phosphodiesterase-5 inhibitor sildenafil prevents neuroinflammation, lowers beta-amyloid levels and improves cognitive

performance in APP/PS1 transgenic mice. *Behav Brain Res* 250, 230-237. doi: 10.1016/j.bbr.2013.05.017.

Zhang, J., Yin, Y., Ji, Z., Cai, Z., Zhao, B., Li, J., et al. (2017). Endophilin2 Interacts with GluA1 to Mediate AMPA Receptor Endocytosis Induced by Oligomeric Amyloid-β. *Neural plasticity* 2017, 8197085-8197085. doi: 10.1155/2017/8197085.

Zhang, T.-T., Shen, F.-Y., Ma, L.-Q., Wen, W., Wang, B., Peng, Y.-Z., et al. (2016). Potentiation of synaptic transmission in Rat anterior cingulate cortex by chronic itch. *Molecular Brain* 9(1), 73. doi: 10.1186/s13041-016-0251-1.

Zhang, W., Wu, J., Ward, M.D., Yang, S., Chuang, Y.-A., Xiao, M., et al. (2015). Structural basis of arc binding to synaptic proteins: implications for cognitive disease. *Neuron* 86(2), 490-500. doi: 10.1016/j.neuron.2015.03.030.

Zhang, X., and Poo, M.M. (2002). Localized synaptic potentiation by BDNF requires local protein synthesis in the developing axon. *Neuron* 36(4), 675-688. doi: 10.1016/s0896-6273(02)01023-1.

Zhang, Y.-P., Holbro, N., and Oertner, T.G. (2008). Optical induction of plasticity at single synapses reveals input-specific accumulation of αCaMKII. *Proceedings of the National Academy of Sciences* 105(33), 12039-12044. doi: 10.1073/pnas.0802940105.

Zhang, Y.-w., Thompson, R., Zhang, H., and Xu, H. (2011). APP processing in Alzheimer's disease. *Molecular Brain* 4, 3-3. doi: 10.1186/1756-6606-4-3.

Zhao, H., Alam, A., San, C.Y., Eguchi, S., Chen, Q., Lian, Q., et al. (2017). Molecular mechanisms of brain-derived neurotrophic factor in neuro-protection: Recent developments. *Brain Res* 1665, 1-21. doi: 10.1016/j.brainres.2017.03.029.

Zhao, J.-P., Murata, Y., and Constantine-Paton, M. (2013). Eye opening and PSD95 are required for long-term potentiation in developing superior colliculus. *Proceedings of the National Academy of Sciences* 110(2), 707-712. doi: 10.1073/pnas.1215854110.

Zhao, L.X., Ge, Y.H., Li, J.B., Xiong, C.H., Law, P.Y., Xu, J.R., et al. (2019). M1 muscarinic receptors regulate the phosphorylation of AMPA receptor subunit GluA1 via a signaling pathway linking cAMP-PKA and PI3K-Akt. *Faseb j* 33(5), 6622-6631. doi: 10.1096/fj.201802351R.

Zhao, M.G., Toyoda, H., Lee, Y.S., Wu, L.J., Ko, S.W., Zhang, X.H., et al. (2005). Roles of NMDA NR2B subtype receptor in prefrontal long-term potentiation and contextual fear memory. *Neuron* 47(6), 859-872. doi: 10.1016/j.neuron.2005.08.014.

Zheng, F., Luo, Y., and Wang, H. (2009). Regulation of brain-derived neurotrophic factor-mediated transcription of the immediate early gene Arc by intracellular calcium and calmodulin. *Journal of neuroscience research* 87(2), 380-392. doi: 10.1002/jnr.21863.

Zheng, H., Jiang, M., Trumbauer, M.E., Hopkins, R., Sirinathsinghji, D.J., Stevens, K.A., et al. (1996). Mice deficient for the amyloid precursor protein gene. *Ann N Y Acad Sci* 777, 421-426. doi: 10.1111/j.1749-6632.1996.tb34456.x.

Zheng, H., Jiang, M., Trumbauer, M.E., Sirinathsinghji, D.J., Hopkins, R., Smith, D.W., et al. (1995). beta-Amyloid precursor protein-deficient mice show reactive gliosis and decreased locomotor activity. *Cell* 81(4), 525-531. doi: 10.1016/0092-8674(95)90073-x.

Zheng, Z., and Keifer, J. (2009). PKA has a critical role in synaptic delivery of GluR1- and GluR4-containing AMPARs during initial stages of acquisition of in vitro classical conditioning. *Journal of neurophysiology* 101(5), 2539-2549. doi: 10.1152/jn.91282.2008.

Zheng, Z., and Keifer, J. (2014). Sequential Delivery of Synaptic GluA1- and GluA4-containing AMPA Receptors (AMPARs) by SAP97 Anchored Protein Complexes in Classical Conditioning. *Journal of Biological Chemistry* 289(15), 10540-10550. doi: 10.1074/jbc.M113.535179.

Zhou, F., Gong, K., Song, B., Ma, T., van Laar, T., Gong, Y., et al. (2012). The APP intracellular domain (AICD) inhibits Wnt signalling and promotes neurite outgrowth. *Biochim Biophys Acta* 1823(8), 1233-1241. doi: 10.1016/j.bbamcr.2012.05.011.

Zhou, Q., Homma, K.J., and Poo, M.-m. (2004). Shrinkage of Dendritic Spines Associated with Long-Term Depression of Hippocampal Synapses. *Neuron* 44(5), 749-757. doi: https://doi.org/10.1016/j.neuron.2004.11.011.

Zhou, X., Hollern, D., Liao, J., Andrechek, E., and Wang, H. (2013). NMDA receptor-mediated excitotoxicity depends on the coactivation of synaptic and extrasynaptic receptors. *Cell Death & Disease* 4(3), e560-e560. doi: 10.1038/cddis.2013.82.

Zhou, Z., Liu, A., Xia, S., Leung, C., Qi, J., Meng, Y., et al. (2018). The C-terminal tails of endogenous GluA1 and GluA2 differentially contribute to hippocampal synaptic plasticity and learning. *Nature Neuroscience* 21(1), 50-62. doi: 10.1038/s41593-017-0030-z.

Zhu, J.J., Esteban, J.A., Hayashi, Y., and Malinow, R. (2000a). Postnatal synaptic potentiation: delivery of GluR4-containing AMPA receptors by spontaneous activity. *Nat Neurosci* 3(11), 1098-1106. doi: 10.1038/80614.

Zhu, J.J., Esteban, J.A., Hayashi, Y., and Malinow, R. (2000b). Postnatal synaptic potentiation: Delivery of GluR4-containing AMPA receptors by spontaneous activity. *Nature Neuroscience* 3(11), 1098-1106. doi: 10.1038/80614.

Zhu, M., Cortese, G.P., and Waites, C.L. (2018). Parkinson's disease-linked Parkin mutations impair glutamatergic signaling in hippocampal neurons. *BMC biology* 16(1), 100-100. doi: 10.1186/s12915-018-0567-7.

Zhuo, M., Hu, Y., Schultz, C., Kandel, E.R., and Hawkins, R.D. (1994). Role of guanylyl cyclase and cGMP-dependent protein kinase in long-term potentiation. *Nature* 368(6472), 635-639. doi: 10.1038/368635a0.

Zingerle, H. (1912). Über einseitigen Schläfenlappendefekt beim Menschen [On a unilateral defect of the temporal lobe in man]. *J. Psychol. Neurol* 18, 205-235.

Zonouzi, M., Renzi, M., Farrant, M., and Cull-Candy, S.G. (2011). Bidirectional plasticity of calcium-permeable AMPA receptors in oligodendrocyte lineage cells. *Nature neuroscience* 14(11), 1430-1438. doi: 10.1038/nn.2942.

Zorumski, C.F., and Izumi, Y. (2012). NMDA receptors and metaplasticity: mechanisms and possible roles in neuropsychiatric disorders. *Neuroscience and biobehavioral reviews* 36(3), 989-1000. doi: 10.1016/j.neubiorev.2011.12.011.

Zou, C., Crux, S., Marinesco, S., Montagna, E., Sgobio, C., Shi, Y., et al. (2016). Amyloid precursor protein maintains constitutive and adaptive plasticity of dendritic spines in adult brain by regulating D-serine homeostasis. *Embo j* 35(20), 2213-2222. doi: 10.15252/embj.201694085.

Zucker, R.S., and Regehr, W.G. (2002). Short-term synaptic plasticity. *Annu Rev Physiol* 64, 355-405. doi: 10.1146/annurev.physiol.64.092501.114547.

Appendix

1. Image analysis

1.1. Quantifying Arc Protein Expression in Primary Hippocampal Neurons

To quantify Arc protein expression throughout somatic and dendritic compartments of cultured hippocampal neurons (refer section 4.1. *Examining Arc Expression in Response to sAPPα*), individual MAP2 and Arc channel images are first imported into ImageJ as *.tif* files. Here, neurons of interest are manually outlined and regions of interest (ROIs) are determined for measurements of neuronal area and integrated density (Figure A-1,2). These measurements are then accounted for by average measurements of mean gray value from close-by selections.

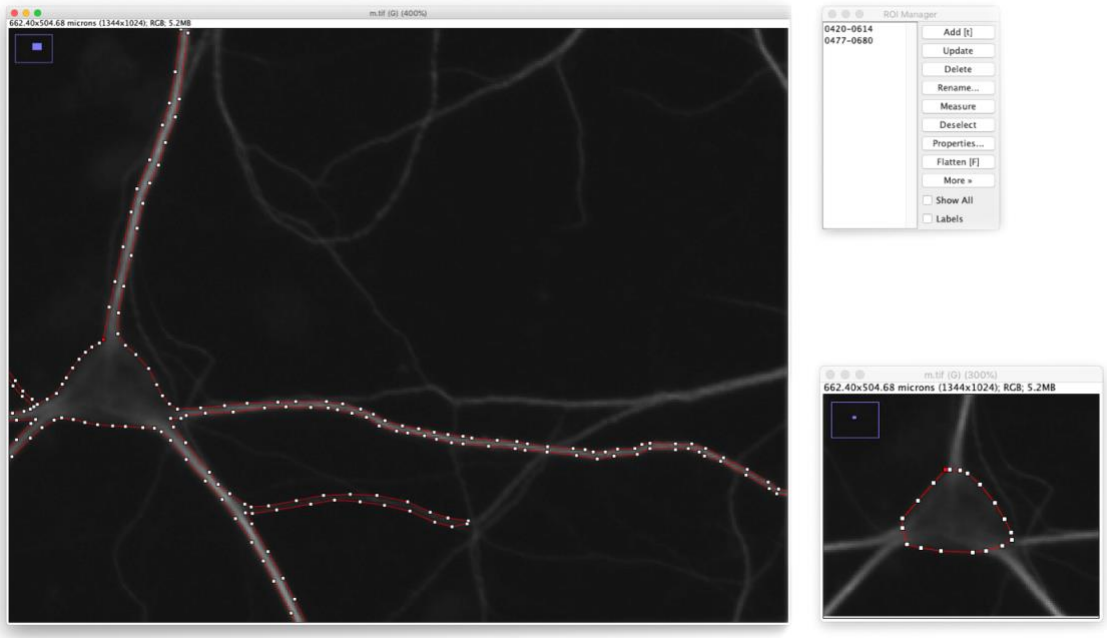


Figure A-1 |Setting of MAP2 mask. Using the MAP2 channel as a guide, an identified neuron and individual soma is traced using the polygon selection tool (◯), and is saved as regions of interest (ROIs). Representative image shows magnified (400%) window of a neuron of interest (left), and somatic compartment (300% magnification, right) with selected ROIs.

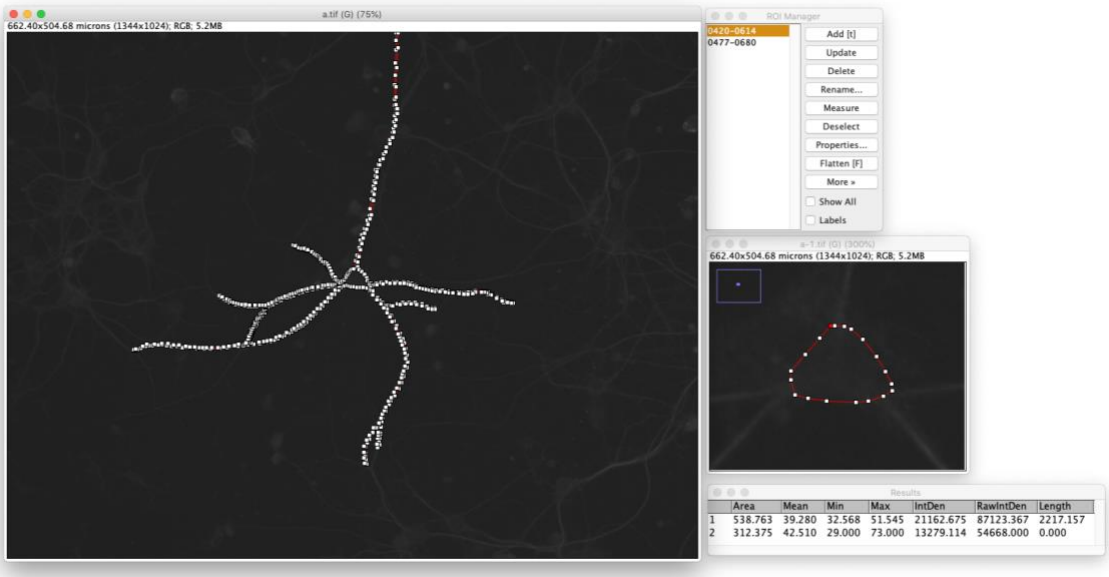


Figure A-2 | Measuring integrated density. Using the masks generated above, measurements including neuron area and integrated density (IntDen) are taken for both whole cell and somatic masks. Representative images show whole cell (75% magnification, left) and somatic (300% magnification, right).

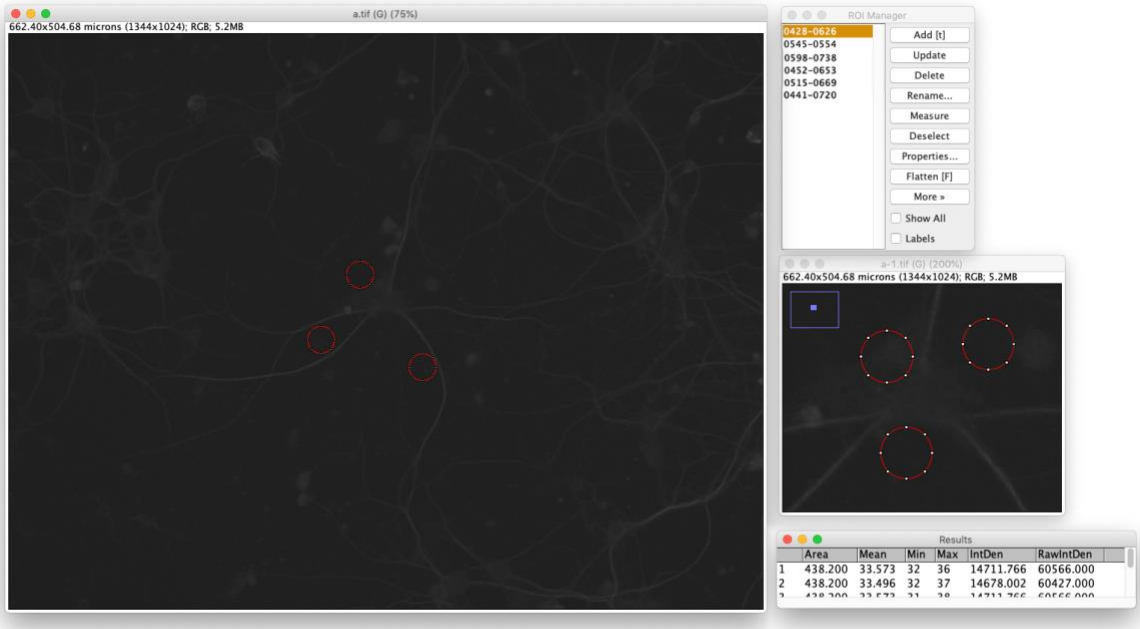


Figure A-3 | Accounting for background fluorescence. Following measurements of integrated density, in order to determined background fluorescence, three circular selections (◯) of equal size are obtained adjacent to both dendritic and somatic compartments, from which the mean gray value is obtained. These values were averaged for each compartment, multiplied by neuron or soma area and subtracted from respective integrated density values for corrected total cell fluorescence (CTCF).

1.2. Quantifying pCREB Protein in Primary Hippocampal Slices

To quantify pCREB expression in hippocampal slices (refer section 4.2. *sAPPα Increases CREB Phosphorylation and Arc Protein in Acute Hippocampal Slices*), a threshold image of the DAPI channel was generated and used to define a ROI within the CA1 area, specific to the cell body layer (Figure A-4). This ROI was then used to define the area to be measured using the integrated density of pCREB

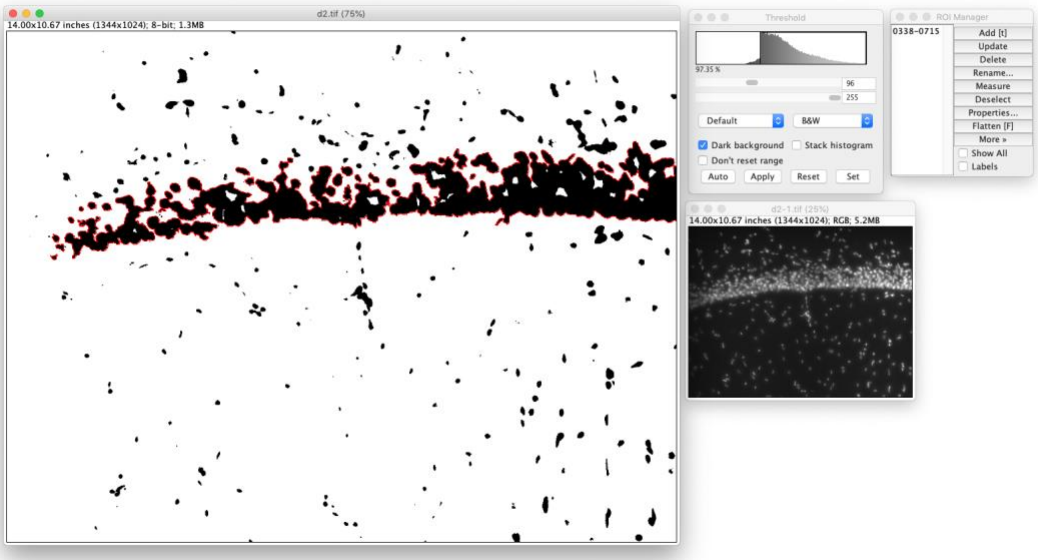


Figure A-4 | Setting the ROI of the cell body layer. The cell body layer of area CA1 is used to define the ROI using the threshold tool in ImageJ.

signal (Figure A-5).

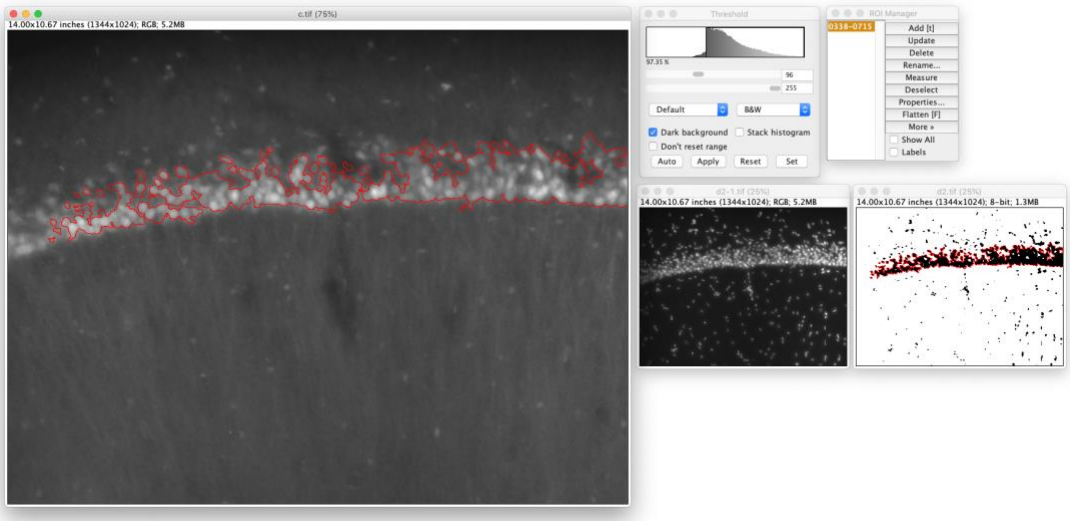


Figure A-5 | Measuring integrated density of the cell body layer. Representative image of pCREB fluorescence, measured using the defined ROI.

1.2.1. Quantifying Arc Protein Signal in Hippocampal Slices

To quantify Arc protein expression, a region of interest was set which encompassed area CA1, CA3 or the DG of all slices quantified. This ROI was used to measure the integrated density of Arc protein fluorescence (Figure A-6).

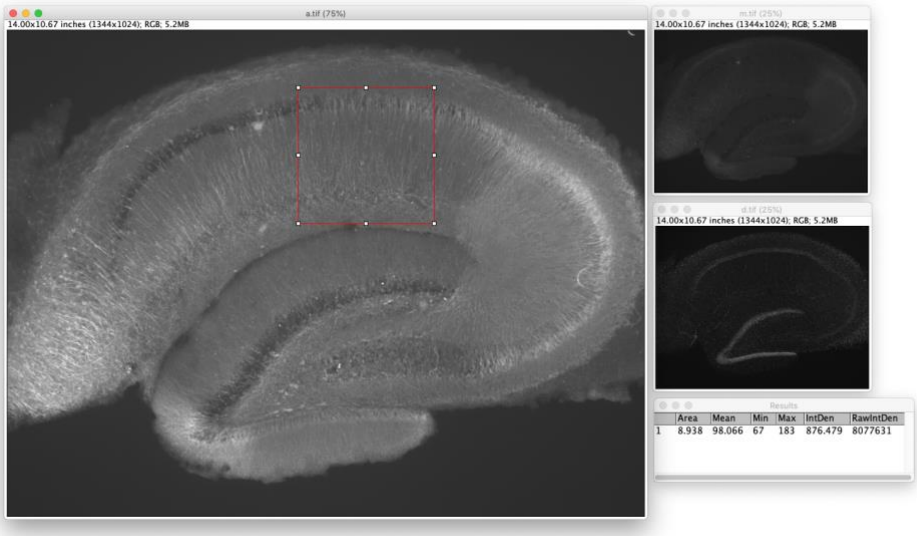


Figure A-6 | Measuring the fluorescence of the CA1 ROI. Representative image of Arc protein fluorescence, measured using the defined ROI

1.3. Quantifying PLA Signal in Primary Hippocampal Neurons

To quantify PLA signal in cultured hippocampal neurons (refer section 6.1. *sAPPα Regulates the Dynamic Control of AMPA Receptors at the Cell Surface*), a custom-made ImageJ macro (Maximilian Heumüller, Schuman Laboratory, Max Planck Institute for Brain Research, as used in (tom Dieck et al., 2015) was used. Using this, a MAP2 mask is generated to determine the area of PLA signal within a neuron of interest. In order to achieve this, the MAP2 mask generated is dilated by 1 pixel to ensure the inclusion of signal in dendritic spines (Figure A-7). RGB *.tif* files are composed from corresponding MAP2, PLA, and DAPI channels, and MAP2 and PLA signals are assigned as mask and signal channels, respectively (Figure A-7). Manually, a threshold for the PLA signal is defined by the average of all images and applied across conditions (Figure A-7). These parameters are thus used throughout the course of the macro in order to determine PLA signal per neuron of interest.

Henceforth, the PLA macro is used to define a MAP2 mask (Figure A-8), isolate the neuron of interest from surrounding neurons (Figure A-9,10), and detection of PLA signal within the defined MAP2 mask (Figure A-10). This allows for quantification of PLA signal per neuron, normalized to the area of the neuron of interest. For the quantification of PLA within somatic and dendritic compartments, individual soma were isolated from dendrites and analysed. Similarly, the proximal 50 µm of dendrites were isolated and straightened using the ImageJ 'straighten' function. Dendrites were then analysed using the PLA quantification macro.

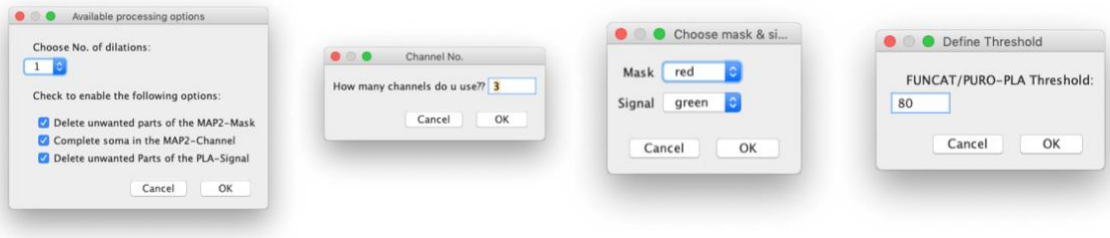


Figure A-7 | Running the PLA quantification macro. Before images are imported, parameters are set including (from left to right); mask signal dilations and appropriate optional steps, the number of channels comprising the RGB image, mask and signal channel assignment, and the previously determined PLA threshold value.

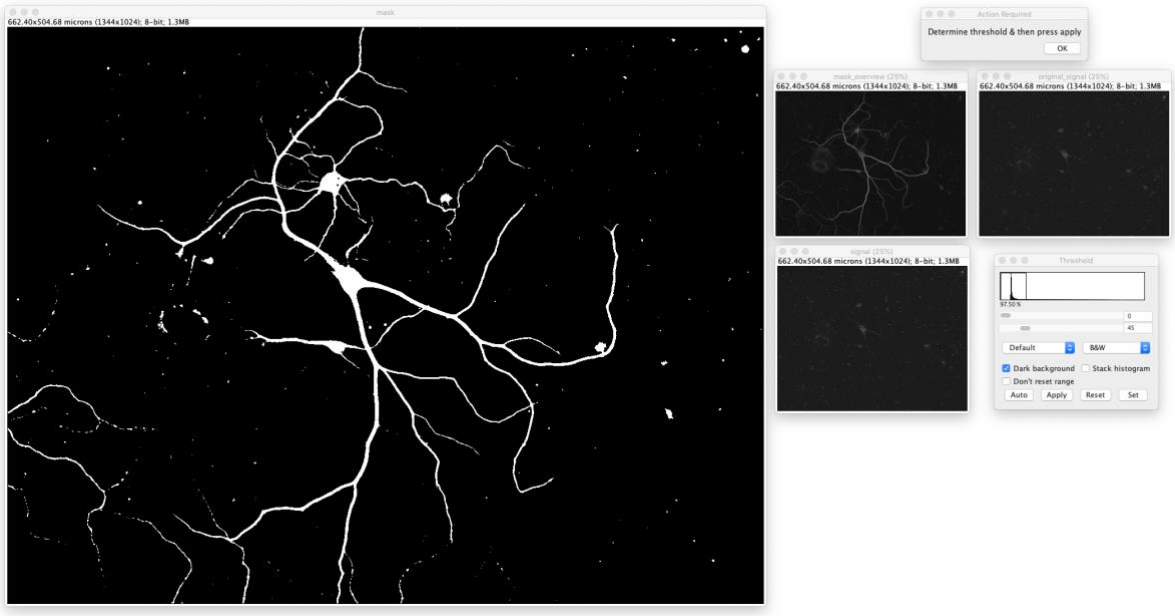


Figure A-8 | Defining the MAP2 threshold and mask. The MAP2 signal threshold was manually defined to create a mask of the neuronal somato-dendritic compartments ( ). The threshold signal is pictured on the left, the raw MAP2 and PLA signal channels are shown on the right. Representative images show the MAP2 mask (left), raw MAP2 signal (top right), and raw PLA signal (top right, bottom right)

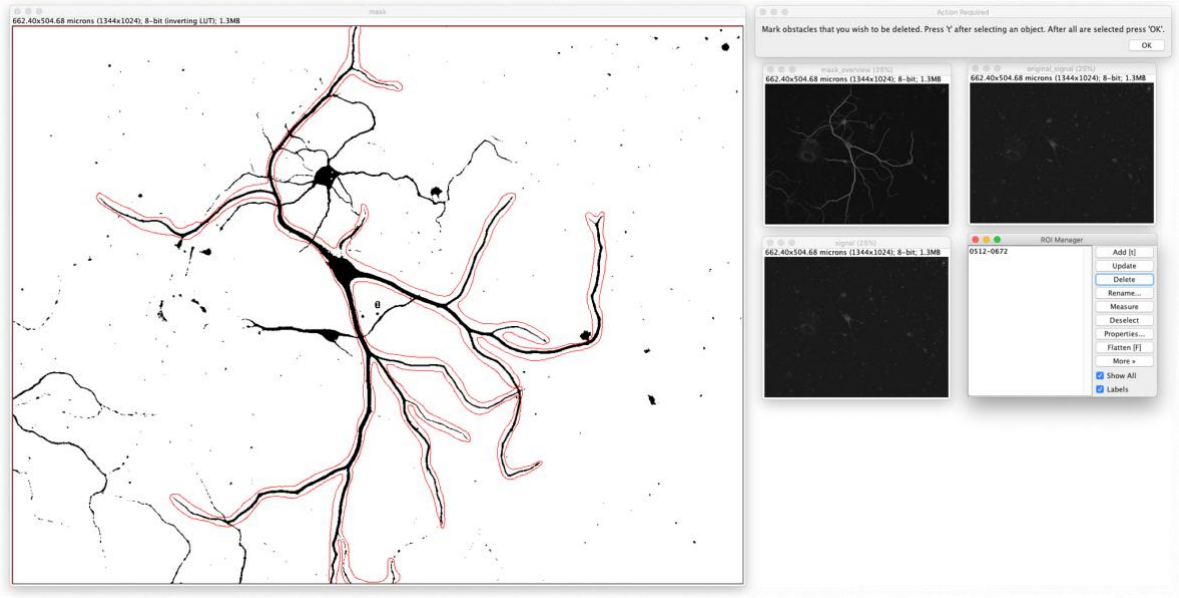


Figure A-9 | Isolation of the cell of interest from surrounding cells. Using the MAP2 mask, the processes and cell body belonging to the target cell are outlined (◯) using the freehand selection tool. Following this, the selection was inverted to select surrounding non-target signal. This is saved as an ROI to be removed by the macro.

Due to the low level of MAP2 in the somatic compartment, some neurons require the soma to be manually defined to create a solid mask, permitting measurement of PLA signal within the soma (Figure A-10,11).

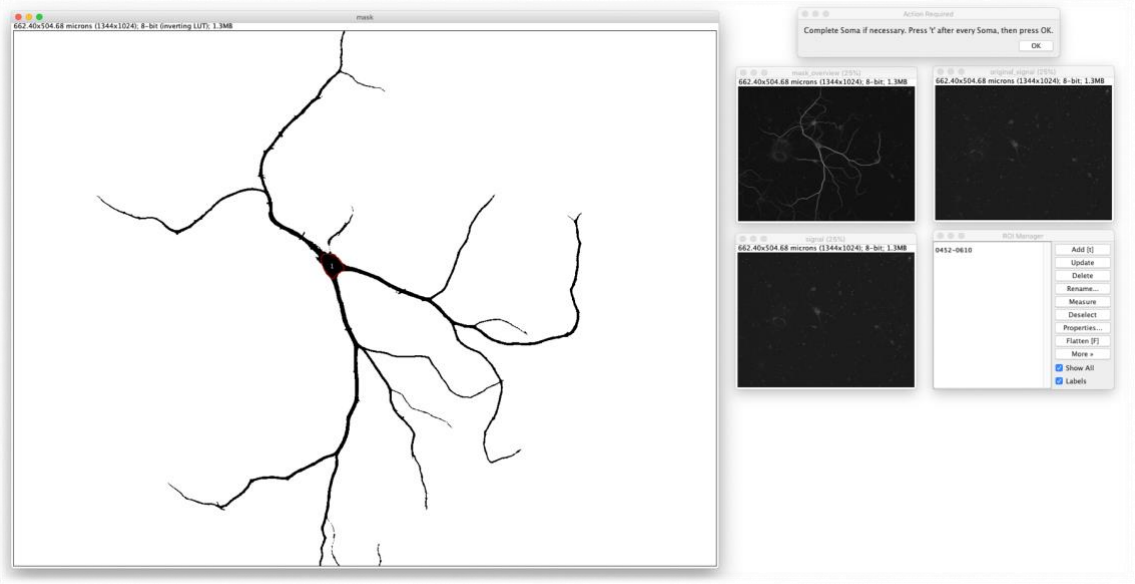


Figure A-10 | Isolated target neuron. Following isolation of the target neuron from all surrounding cells, the constraints of the somatic compartment are defined, where necessary as a ROI, using the raw MAP2 signal as a guide

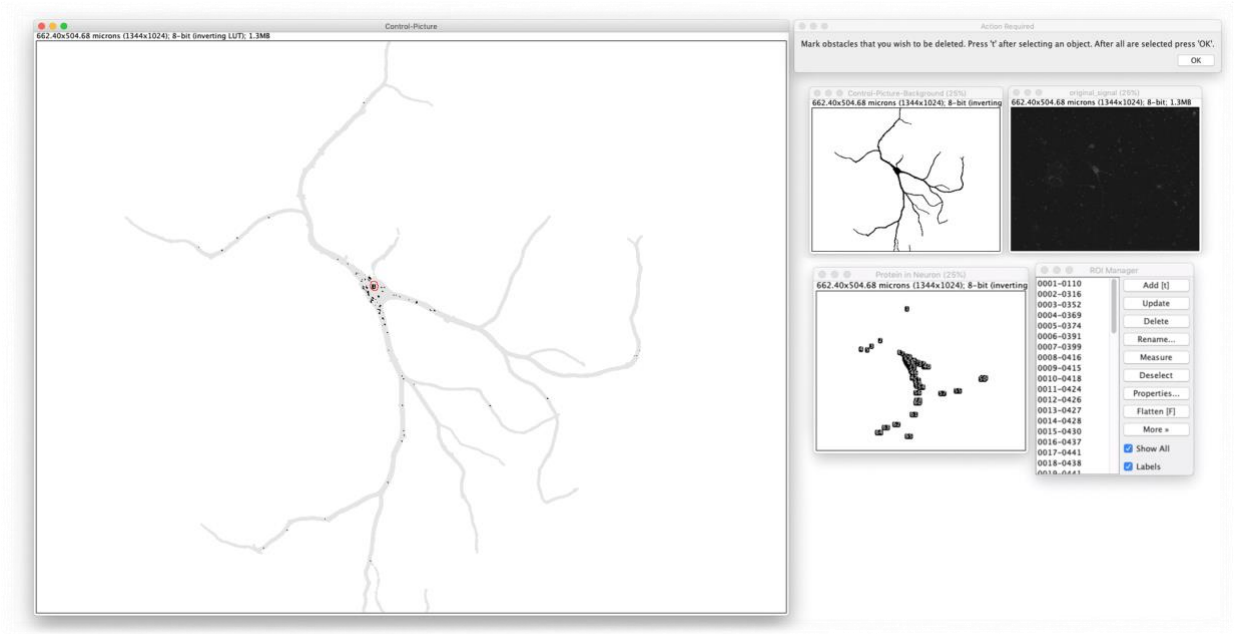


Figure A-11 | Detection and quantification of PLA signal. The final step includes verification of the PLA signal, and an option to delete any signal deemed as either noise or non-neuronal. Following this, the macro calculates the PLA signal area within the MAP2-defined mask, and is divided by the non-dilated MAP2 area. Representative images show the MAP2 mask (left, ■), PLA signal (■), and removed PLA puncta (○), defined MAP2 mask (top right), raw PLA signal (far right), PLA signal mapped by ROI (bottom left), and ROI manager of identified PLA signal (bottom right).

1.4. Quantifying Colocalization of PLA Signal Within Synapses

In order to quantify the PLA signal within synapses the ImageJ plugin 'Just Another Colocalization Plugin' (JACoP) was used (Figure A-12). Before analysis within ImageJ, the threshold value of both PLA and Synapsin-1 signal is generated to be using during JACoP analysis. As shown by Figure A-13, threshold values are entered into JaCoP and a Mander's overlap coefficient is calculated, generating a value proportional to the level of B (PLA) overlapping A (synapsin-1). Following JACoP analysis, the centre of the PLA was determined as the brightest pixel and used to measure distance to the nearest synapsin-1 centre of mass (Figure A-14).

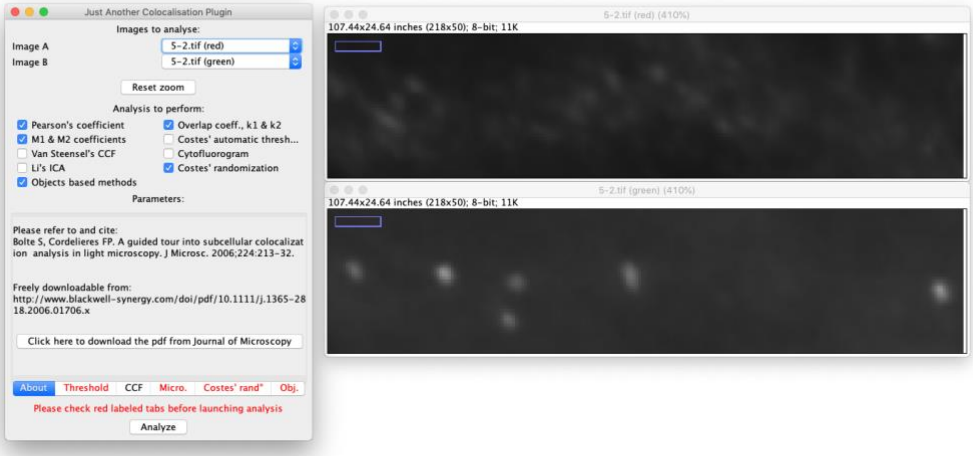


Figure A-12 | Running JACoP. Synapsin-1 (upper panel) and PLA (lower panel) signal channels are split and JACoP is run with the settings pictured above (left panel).

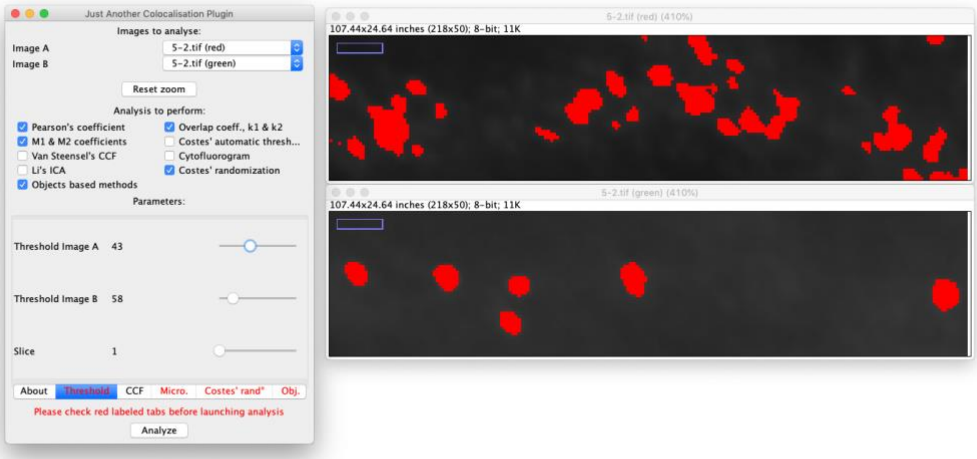


Figure A-13 | Setting of the threshold values. Previously determined threshold values were input into JACoP to generate a mask (■) for both PLA and synapsin-1 signal.

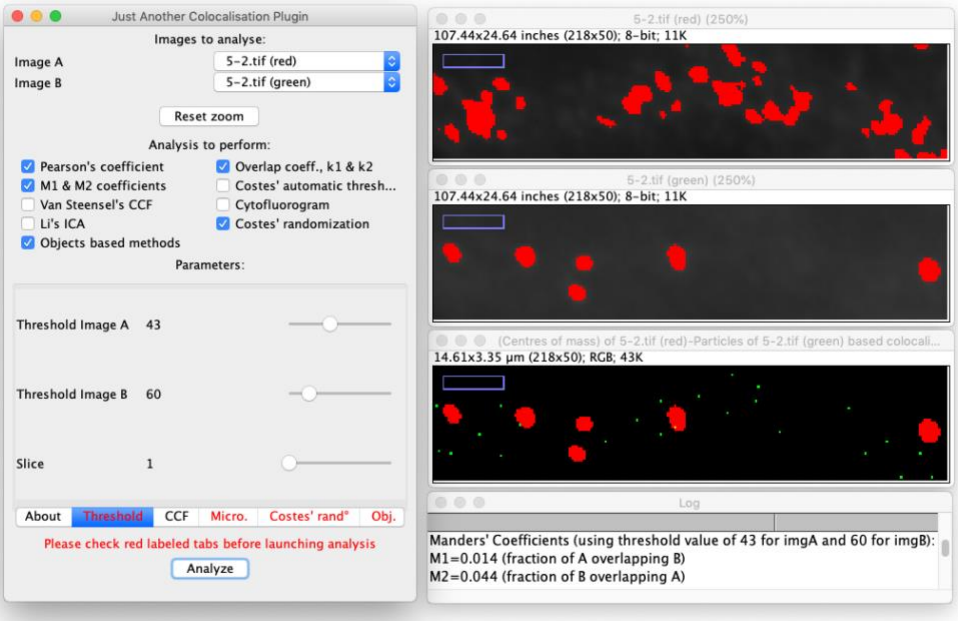


Figure A-14 | Measuring distance to synapse centre. JACoP generates a Mander's Coefficient (fraction of B (PLA) overlapping A (synapsin-1; lower panel) and representative image of center of mass of Synapsin-1 and PLA signal (third panel).

As described in section 3.1.3. *Cellular Populations Within Primary Hippocampal Cell Cultures*, the health of cells in culture can be determined by observation of MAP2 immunoreactivity. In response to insult, cultured neurons generate increased free radicals, which in turn promotes lipid peroxidation (Catlin et al., 2016). This presents as characteristic neuritic blebbing, fragmented dendrites, and swollen cell bodies as indicated in Figure A-15.

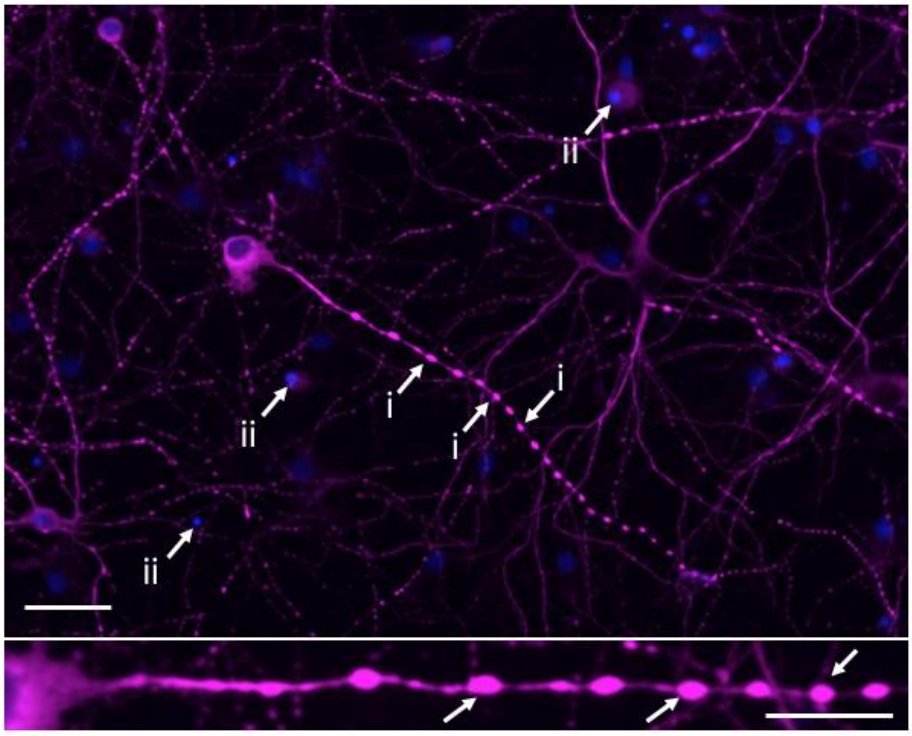


Figure A-15 | Representative image of an unhealthy hippocampal neuron. Image shows MAP2 (■), and DAPI (■). Arrows (i) indicate sites of serious membrane fragmentation (blebbing) in the absence of strong continuous MAP2-positive staining, (ii) shows populations of condensed nuclei. Inset image shows magnified proximal dendritic segment, arrows indicate sites of blebbing. Scale bar = 50 µm (A), inset image = 10 µm.

3. Accell™ siRNA Specificity

Within the last ten years, gene silencing by small interfering RNAs (siRNAs) has emerged as a promising method for inhibiting the expression of target genes and proteins (Akhtar and Benter, 2007; de Fougerolles et al., 2007). Alternative to complete gene knockout animals (Bevan, 2010), siRNAs serve as an effective, inexpensive method for inhibiting gene expression both *in vitro* and *in vivo* (Mocellin and Provenzano, 2004). Unfortunately, naked siRNAs are relatively unstable due to the presence of endo- and exonucleases (Bartlett and Davis, 2007). Therefore, various delivery systems have been developed to circumvent this problem, including liposomes (Pulford et al., 2010), viruses (Dreyer, 2010), chemical modifications (Walton et al., 2010), electroporation (Zhao et al., 2005), exosomes (Alvarez-Erviti et al., 2011), and conjugation to lipid based reagents (i.e. Lipofectamine 2000)(Chen et al., 2009). Recently, Thermo Scientific Dharmacon have developed Accell™ siRNA, a chemically modified siRNA which allows for passive delivery of antisense oligonucleotides in the absence of viral- or lipid-based transfection agents, increasing functionality and stability of the interfering RNA.

Endogenous siRNA, and synthetic Accell™ siRNA both consist initially of a double stranded RNA oligonucleotide bearing both a 5' phosphate (on the antisense strand) and 3' double uracil (3'-UU) overhang. When incorporated exogenously into the cell of interest, double-stranded siRNA is separated and the 5' antisense single-stranded RNA binds the multiprotein component complex RISC (RNA-induced silencing complex). Here, the antisense RNA guides and aligns the RISC complex on the target mRNA to allow for cleavage and degradation of the target mRNA (Bartel, 2009; Kim et al., 2009; Ketting, 2011; Horizon Discovery, 2014; Dana et al., 2017).

In order to first assess the efficacy of Accell™ siRNA in our hippocampal cell cultures (refer sections 6.1.4. *Accell™ Arc siRNA Inhibits Somatic and Dendritic sAPPα-Dependent, but not Basal, Arc Expression*, 6.1.5. *Internalization of de novo GluA1 is Dependent on Arc Expression* and 6.1.6. *Non-Targeting siRNA has no Significant Effect on de novo Cell Surface GluA1*), we utilized fluorescent Accell™ red non-targeting control siRNA for visualization of siRNA expression by conventional epifluorescence microscopy. As shown by Figure A-16, treatment of cultures with the fluorescent Accell™ siRNA across an equivalent treatment duration of our experimental conditions, resulted in clear expression within MAP2-positive neurons in both the soma and dendrites. Interestingly, cells present in culture which appeared negative for MAP2 expressed the highest levels of fluorescent Accell™ siRNA. These populations may be indicative of glial cells, such as astrocytes, microglia, or oligodendrocytes.

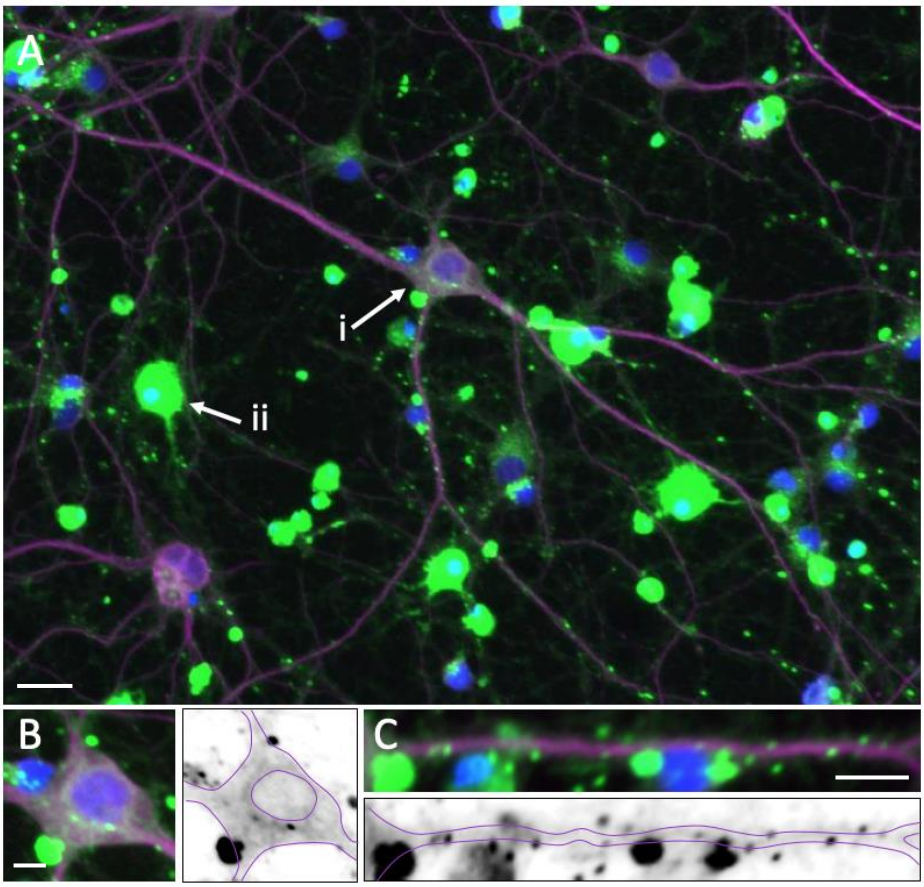


Figure A-16 | Specificity of Accell™ siRNA expression. A) Immunocytochemistry showed populations of i) neurons (MAP2-positive, ■) expressing fluorescent Accell™ siRNA (■). ii) cells negative for MAP2 but positive for DAPI (■) also appear to express Accell™ siRNA. Lower panels (B & C) show magnified somatic and dendritic compartments, respectively, and corresponding black & white Accell™ siRNA signal. Scale bars = 50 µm (A), 10 µm (B & C).

4. Antibody Specificity

Here we have utilized western blotting as a method to validate the specificity and reactivity of the antibodies used in a majority of the experiments described. Protein was extracted from primary cortical cultures and processed for western blot as described in section 2.4.3. *Western Blot*. The highly abundant protein αTubulin (55 kDa) was used as a loading control. We then probed for the proteins Arc (55 kDa), and GluA1 (100 kDa) using antibodies targeted towards both C- and N-terminal epitopes (Figure A-17).

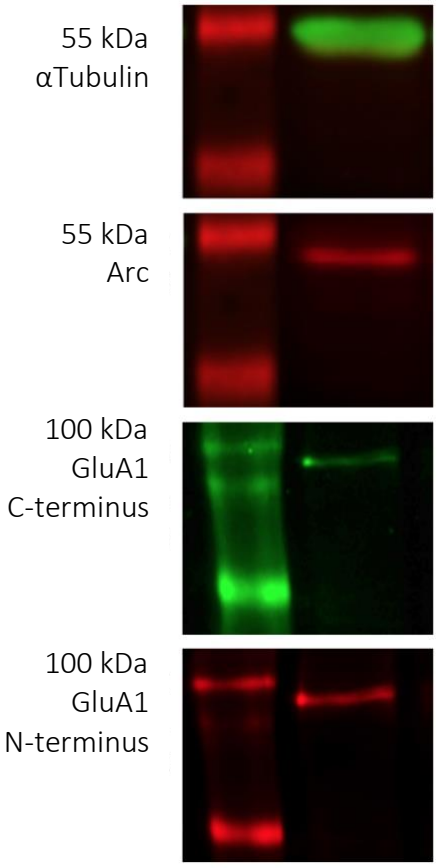


Figure A-17 | Specificity of Primary Antibodies. Representative image of western blots showing immunoreactivity for the loading control anti-αTubulin (upper panel; 50 kDa; IRDye800, ■), anti-Arc (second panel, 45 kDa; IRDye680, ■), GluA1 detected using a C-terminal antibody (third panel 100 kDa; IRDye800, ■), and GluA1 detected using an N-terminal antibody (lower panel, 100 kDa; IRDye680, ■).

Tubulin and Arc western blots were provided by Honours student Maya Barrett. GluA1 western blots provided by Honours student Courteney Westlake.