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The integrated role of ACh, ERK and mTOR in the mechanisms of hippocampal inhibitory avoidance memory

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Abstract

The purpose of this review is to summarize the present knowledge on the interplay among the cholinergic system, Extracellular signal-Regulated Kinase (ERK) and Mammalian Target of Rapamycin (mTOR) pathways in the development of short and long term memories during the acquisition and recall of the step-down inhibitory avoidance in the hippocampus. The step-down inhibitory avoidance is a form of associative learning that is acquired in a relatively simple one-trial test through several sensorial inputs. Inhibitory avoidance depends on the integrated activity of hippocampal CA1 and other brain areas. Recall can be performed at different times after acquisition, thus allowing for the study of both short and long term memory. Among the many neurotransmitter systems involved, the cholinergic neurons that originate in the basal forebrain and project to the hippocampus are of crucial importance in inhibitory avoidance processes. Acetylcholine released from cholinergic fibers during acquisition and/or recall of behavioural tasks activates muscarinic and nicotinic acetylcholine receptors and brings about a long-lasting potentiation of the postsynaptic membrane followed by downstream activation of intracellular pathway (ERK, among others) that create conditions favourable for neuronal plasticity. ERK appears to be salient not only in long term memory, but also in the molecular mechanisms underlying short term memory formation in the hippocampus. Since ERK can function as a biochemical coincidence detector in response to extracellular signals in neurons, the activation of ERK-dependent downstream effectors is determined, in part, by the duration of ERK phosphorylation itself. Long term memories require protein synthesis, that in the synapto-dendritic compartment represents a direct mechanism that can produce rapid changes in protein content in response to synaptic activity. mTOR in the brain regulates protein translation in response to neuronal activity, thereby modulating synaptic plasticity and long term memory formation. Some studies demonstrate a complex interplay among the cholinergic system, ERK and mTOR. It has been shown that co-activation of muscarinic acetylcholine receptors and β -adrenergic receptors facilitates the conversion of short term to long term synaptic plasticity through an ERK- and mTOR-dependent mechanism which requires translation initiation. It seems therefore that the complex interplay among the cholinergic system, ERK and mTOR is crucial in the development of new inhibitory avoidance memories in the hippocampus.

Keywords: Memory, hippocampus, acetylcholine, ERK, mTOR, Inhibitory avoidance

Abbreviations

4E-BPs: 4E binding proteins

ACh: Acetylcholine

AD: Alzheimer's disease

APP: Amyloid precursor protein

CaMKII: Ca²⁺/calmodulin-dependent protein kinase II

CREB: cAMP response element-binding protein

DG: Dentate gyrus

eEF1A: eukaryotic Elongation Factor 1A

eEF2: eukaryotic Elongation Factor 2

ERK: Extracellular signal-regulated kinase

GABA: gamma-aminobutyric acid

GPCRs: G protein-coupled receptors

IA: Inhibitory avoidance

ICV: intracerebroventricular

IP: intraperitoneal

JNK: c-Jun N-terminal kinase

LTM: Long Term Memory

LTP: Long term potentiation

mAChRs: Muscarinic acetylcholine receptors

M1,...,M5: Muscarinic receptor 1,...,5

MAP: Microtubule-associated proteins

MAPK: Mitogen activated protein kinase

MEK: Mitogen-activated protein kinase kinase

mTOR: Mammalian Target of Rapamycin

mTORC1: mTOR Complex1

nAChRs: Nicotinic acetylcholine receptors

NBM: nucleus basalis magnocellularis

NMDA: N-methyl-D-aspartate

p38MAPK: p38 Mitogen Activated Protein Kinase

p70S6K: p70 ribosomal subunit S6 Kinase

PKA: Protein Kinase A

PKC: Protein Kinase C

STM: Short Term Memory

TgCRND8: Transgenic Centre for Research in Neurodegenerative Diseases 8

wt: wild type

1. Introduction

As St. Augustine wrote in the “Confessions” in the IVth Century a.d. *“And I come to the fields and spacious palaces of my memory, where are the treasures of innumerable images, brought into it from things of all sorts perceived by the senses. ... Nor yet do the things themselves enter in; only the images of the things perceived are there in readiness, for thought to recall. Which images, how they are formed, who can tell, though it doth plainly appear by which sense each hath been brought in and stored up?”* (St. Augustine, 398). The purpose of this review is to try to answer to the question that already fascinated St. Augustine on how memories are formed, by summarizing some of the present knowledge on the mechanisms that underlie memory development in our brain.

The formation of memories is the result of cellular and molecular mechanisms activated in different structures of the brain. The ability of an animal to adapt its behaviour in response to environmental stimuli depends on the structural and functional plasticity of several brain regions. Therefore, it is of the utmost importance to understand how and where in the brain experiences are encoded into lasting memories.

A single learning experience starts a cascade of events, which can lead to different forms of memory: short-term memory (STM) that lasts minutes to hours and long term memory (LTM) that lasts days, weeks, and even a lifetime (McGaugh, 1966). A major question of memory neurobiology is whether these two forms are related or independent phenomena. Some cellular mechanisms that underlie the development of STM overlap with those of LTM, but other mechanisms are independent (Izquierdo et al., 1998a; Izquierdo, Medina, Vianna, Izquierdo, & Barros, 1999; Izquierdo et al., 2002). A unique characteristic of LTM is the need for a consolidation period during which synaptic, structural, and functional modifications occur (Igaz, Vianna, Medina, & Izquierdo, 2002). The most important is protein synthesis on which LTM, but not STM, depends (Davis & Squire, 1984; Freeman, Rose, & Scholey, 1995; Tiunova, Anokhin, Rose, & Mileusnic, 1996; Bourtchouladze et al., 1998; Schafe, Nadel, Sullivan, Harris, & LeDoux, 1999; Quevedo et al., 1999).

Memory is not a unitary function. Memory depends on the integrated activity of various brain structures and neurotransmitter systems and involves multiple receptors, postsynaptic mechanisms, and signal transduction pathways (Izquierdo et al., 1998a). Among the various brain structures implicated in memory formation, the CA1 region of the hippocampus plays a major role in memory encoding (Squire, 1992; Hasselmo, Wyble, & Wallenstein, 1996; Vinogradova, 2001; Eichenbaum, 2001; Lisman & Grace, 2005).

Step-down inhibitory avoidance memory

The step-down inhibitory avoidance (IA) is a form of associative learning that is acquired in one trial through several sensory inputs. IA memory depends on the integrated activity of CA1, entorhinal and posterior parietal cortex, and is modulated by the amygdala and by the basal forebrain cholinergic neurons of the medial septum and indirectly by stress hormones (Izquierdo, 1989; Izquierdo & Medina, 1997; Cammarota, Bevilaqua, Medina, & Izquierdo, 2007). The step-down IA is a widely used task in memory studies (McGaugh, 1966; Gold, 1986; McGaugh & Izquierdo, 2000; Izquierdo et al., 2007) and relies upon the natural tendency of an animal to explore a novel environment. In the IA acquisition task, the animal is placed on an elevated platform by one wall of an arena, steps down to explore the arena and learns to associate exploration with a punishment (a foot shock delivered through the floor grid). On a subsequent exposure to the same environment (recall task), the animal increases the latency to step down onto the floor grid, or avoids stepping on the grid. The natural exploratory behaviour is repressed after the punishment is given, without affecting the exploratory behaviour while on the safe, non-aversive part of the training apparatus. IA is an emotionally-arousing paradigm (Izquierdo et al., 1997; Maren, 2001), that involves:

- i) an explicit, associative component to the context,
- ii) an operant-like conditioning component to the shock, since the animal may avoid the aversive stimulus (Wilensky, Schafe, & LeDoux, 2000),
- iii) a spatial memory component, since the animal remembers the location where the noxious stimulus was given during acquisition (Cammarota, Bevilaqua, Medina, & Izquierdo, 2007).

In the IA, the environment is arranged so that the animal can avoid a painful stimulus; i.e., the “escape” or avoidance is an option available to an animal that could learn and perform it. From an experimental view point, IA is a relatively simple test since it is acquired in a one-trial session. Recall can be performed at different times after acquisition, thus allowing to study both STM (Izquierdo et al., 1998a; Izquierdo et al., 1998b) and LTM mechanisms (Izquierdo et al., 2002).

IA depends upon the activation of the cholinergic system, since its acquisition is impaired by pre-training (Izquierdo et al., 1998b; Giovannini, Bartolini, Bacciottini, Greco, & Blandina, 1999) or post-training peripheral administration of mAChRs antagonists (Table 1) (Izquierdo et al., 1998b; Giovannini, Bartolini, Bacciottini, Greco, & Blandina, 1999; McGaugh & Izquierdo, 2000), and is facilitated by mAChRs agonists (Baratti, Huygens, Mino, Merlo, & Gardella, 1979; Barros, Pereira, Medina, & Izquierdo, 2002).

Short term and long term memory mechanisms: open questions

All types of novel stimuli induce the activation of the forebrain cholinergic system (Pepeu and Giovannini, 2006). In this review we shall examine how the cholinergic system participates in the formation of STM and LTM in CA1 during the acquisition and performance of the step-down inhibitory avoidance task in the rat. A key question that still remains unanswered is whether STM represents a step toward LTM only or the formation of the two memory types reflects separate processes.

According to current hypotheses, STM and LTM formation imply biochemical processes that act in parallel and on different time scales (Izquierdo et al., 1998a; Izquierdo, Medina, Vianna, Izquierdo, & Barros, 1999; Izquierdo et al., 2002). Nevertheless, to better answer to this question, it is necessary to demonstrate that STM can be suppressed without affecting LTM. The pharmacology and molecular bases of IA have been studied by us (Giovannini et al., 2005; Lana et al., 2013) and Izquierdo's group, particularly in the CA1 region (Igaz, Bekinschtein, Izquierdo, & Medina, 2004; Marti, Ramirez, Dos Reis, & Izquierdo, 2004). Moreover, for the reasons mentioned above, unlike multitrial learning tasks, IA offers the possibility to neuroscientists to distinguish the processes involved in STM and LTM by the simple modulation of time parameters after the acquisition task. In particular we shall try to shed light on the complex interplay among the cholinergic system, ERK and mTOR in IA memory formation. Among the several actors downstream of the cholinergic activation implicated in STM and LTM formation, this review will focus particularly on ERK and mTOR since they can modulate both early processes such as phosphorylation of protein substrates, implicated in STM, and later processes like immediate or *de novo* proteosynthesis in neurons, implicated in LTM formation (Davis & Squire, 1984; Freeman, Rose, & Scholey, 1995; Tiunova, Anokhin, Rose, & Mileusnic, 1996; Atkins, Selcher, Petraitis, Trzaskos, & Sweatt, 1998; Bourtchouladze et al., 1998; Quevedo et al., 1999; Schafe, Nadel, Sullivan, Harris, & LeDoux, 1999; Cammarota et al., 2000; Alonso, Viola, Izquierdo, & Medina, 2002; Tsokas, Ma, Iyengar, Landau, & Blitzer, 2007; Myskiw et al., 2008).

2. The hippocampal cholinergic system in learning and memory

Among the many neurotransmitter systems, the cholinergic fibres that originate in the basal forebrain and project to the hippocampus are of crucial importance in learning and memory processes (Zola-Morgan & Squire, 1993; Muir, Everitt, & Robbins, 1996; Everitt & Robbins, 1997; Sarter & Bruno, 1997a; Sarter & Bruno, 2000). The hippocampus receives a large cholinergic input (Frotscher & Leranth, 1985) from neurons located in the medial

septum and the vertical limb of the diagonal band of Broca, denominated by Mesulam Cholinergic sector 1 (Ch1) and Cholinergic sector 2 (Ch2) (Mesulam, Mufson, Vainer, & Levey, 1983). These neuronal clusters are parts of the forebrain cholinergic system, formed by an aggregate of discontinuous islands of multipolar cells with extensive dendritic trees. An analysis of the targets of the cholinergic fibers shows that pyramidal cells, granule cells, and non-pyramidal neurons of the hippocampus receive cholinergic input (Frotscher & Leranth, 1985). ACh, released from the cholinergic terminals, impinges on hippocampal muscarinic and nicotinic ACh receptors. As described later in the chapter, ACh receptors modify neuronal activity, through multiple signalling cascades characterized by different spatial location and time course (Teles-Grilo Ruivo LM & Mellor JR, 2013).

Muscarinic ACh receptors

The muscarinic ACh receptors (mAChRs) are members of the class of heptahelical G protein-coupled receptors (GPCRs). Five main subtypes of muscarinic receptors (M1–M5) have been identified. Their localization in the hippocampal formation was investigated using subtype-specific antibodies (Levey, Edmunds, Hersch, Wiley, & Heilman, 1995). Each receptor subtype, differentially localized in the hippocampal areas, modulates a variety of processes, including long term synaptic plasticity (Origlia et al., 2006). M1 receptors are widely expressed on the somata and dendrites of the pyramidal neurons of CA1-CA3 areas and on granule cells of the dentate gyrus. Some M3 receptors are located on pyramidal neurons, on the neuropil of the stratum lacunosum moleculare and the outer third of the molecular layer of dentate gyrus; M2 and M4 subtypes are located presynaptically in several bands of fibers, and postsynaptically in non-pyramidal neurons and in the inner layer of the molecular layer. As a consequence of their pre- and postsynaptic location, mAChRs have different impacts on neuronal activity. Presynaptic mAChRs (M₂, M₄) are coupled to G_{i/o} and inhibit voltage-gated Ca²⁺ channels, decrease cAMP-mediated signaling and inhibit neurotransmitter release at cholinergic (Vannucchi & Pepeu, 1995; Vannucchi, Scali, Kopf, Pepeu, & Casamenti, 1997; Zhang et al., 2002), GABAergic and glutamatergic terminals (Russo, Marchi, Andrioli, Cavazzani, & Raiteri, 1993; Gonzales, Pare, Wichmann, & Smith, 2013; Szabo, Holderith, Gulyas, Freund, & Hajos, 2010; Dasari & Gullledge, 2011). Postsynaptic mAChRs (M₁, M₃, M₅) are coupled to G_{q/11} and potentiate NMDA currents (Markram & Segal, 1990c; Marino, Rouse, Levey, Potter, & Conn, 1998; Fernandez De Sevilla, Nunez, Borde, Malinow, & Buno, 2008), modulate voltage-dependent Ca²⁺ currents (Toselli, Lang, Costa, & Lux, 1989) and activate phospholipase C, inositol trisphosphate and

increase of intracellular Ca^{2+} concentration (Power & Sah, 2002; Gullledge & Kawaguchi, 2007). Furthermore, mAChRs coupled to $G_{q/11}$ inhibit K^+ conductances, causing membrane depolarization and increasing input resistance (Brown & Adams, 1980; Halliwell & Adams, 1982; Cole & Nicoll, 1984; Madison, Lancaster, & Nicoll, 1987; Buchanan, Petrovic, Chamberlain, Marrion, & Mellor, 2010; Giessel and Sabatini, 2010).

The literature on the disruptive effect of muscarinic antagonists, namely scopolamine and atropine, on cognitive processes is extensive and has been largely reviewed (Izquierdo, 1989; Klinkenberg & Blokland, 2010; Brown, 2010; Graef, Schoknecht, Sabri, & Hegerl, 2011). We shall only focus on some examples taken from the literature that corroborate the role of the basal forebrain cholinergic neurons innervating the hippocampus on IA memory formation (Table 1). In several IA studies (Wiener and Messer, 1973; Rush, 1988; Quirarte et al., 1994; Nomura, Nishiyama, Saito, & Matsuki, 1994, Eidi, Zarrindast, Eidi, Oryan & Parivar, 2003; Giovannini et al., 2005; Lana et al., 2013) it has been demonstrated that systemic, intracerebral or intrahippocampal administration of scopolamine before training is effective in impairing recall at 1 h or 24 h after training (Table 1). Nevertheless, the level of shock intensity interferes with the effect of scopolamine on passive avoidance retention, as shown by Quirarte et al. (1994). A dose of 8 mg/kg, IP caused amnesia using low foot shock conditions, but it was not effective when high level of foot shock was employed. Furthermore, intra-hippocampal administration of the muscarinic agonist oxotremorine or of the muscarinic toxin MT2, a highly selective agonist for M1 receptors from the venom of the snake *Dendroaspis angusticeps*, enhances retention of an inhibitory avoidance (Izquierdo et al. 1992; Jerusalinsky, Cervenansky, Walz, Bianchin, & Izquierdo, 1993). These effects can be antagonized by scopolamine (Jerusalinsky, Cervenansky, Walz, Bianchin, & Izquierdo, 1993), and led the authors to postulate that the m1 receptor of the dorsal hippocampus is directly involved memory formation of this task (Jerusalinsky et al., 1993).

Nicotinic ACh receptors

The nicotinic ACh receptors (nAChRs) are a family of ACh-gated ion channels formed by different subtypes, each with specific anatomical distribution as well as different pharmacology and physiology. Twelve neuronal subunits have been described including 9 α ($\alpha 2$ - $\alpha 10$) and 3 β ($\beta 2$ - $\beta 4$) subunits. Only the α subunits contain the binding site for ACh (Alkondon & Albuquerque, 1993). The combination of these subunits defines the function and affinity of the receptor for specific ligands (Sudweeks & Yakel, 2000). In the hippocampus, $\alpha 7$, $\alpha 4\beta 2$ and $\alpha 3\beta 4$ nAChRs have been detected (Teles-Grilo Ruivo LM &

Mellor JR, 2013). The $\alpha 4\beta 2$ subtype is present on the somata of excitatory neurons and presynaptically on GABAergic terminals. The $\alpha 3\beta 4$ subtype was found on glutamatergic and GABAergic terminals. The $\alpha 7$ receptors are located presynaptically and postsynaptically at both glutamatergic and GABAergic synapses and postsynaptically at cholinergic synapses (Ji & Dani, 2000; Alkondon & Albuquerque, 2004). The $\alpha 7$ nAChR, in addition to its ionotropic activity, is associated with metabotropic activity coupled to Ca^{2+} -regulated second messenger signalling (Berg & Conroy, 2002). Activation of nAChRs results in direct Ca^{2+} influx through the channel pore and rapid membrane depolarization. The precise Ca^{2+} permeability of receptors depends on the subunit composition with $\alpha 7$ being the most permeable. Ca^{2+} accumulation in presynaptic terminals facilitates neurotransmitter release (Lena, Changeux, & Mulle, 1993; McGehee, Heath, Gelber, Devay, & Role, 1995; Wonnacott, 1997; Fu, Liou, & Chen, 1998; Tang et al., 2011). Postsynaptically, cations flux through nAChRs mediates fast excitatory synaptic responses (Frazier et al., 1998; McQuiston & Madison, 1999; Ji & Dani, 2000; Alkondon & Albuquerque, 2001; Kawai, Zago & Berg, 2002; Wanaverbecq, Semyanov, Pavlov, Walker, & Kullmann, 2007; Bell, Shim, Chen, & McQuiston, 2011; Gu & Yakel, 2011; Tang et al., 2011). Fast membrane depolarization triggers activation of voltage-gated Ca^{2+} channels, increase of second messenger cAMP (Margiotta, Berg, & Dionne, 1987; Sargent, 1993) and Ca^{2+} release from intracellular stores (Vijayaraghavan, Pugh, Zhang, Rathouz, & Berg, 1992; Sharma & Vijayaraghavan, 2003). Ca^{2+} influx through nAChRs activates Ca^{2+} -dependent Cl^- conductances (Mulle, Choquet, Korn, & Changeux, 1992; Vernino, Amador, Luetje, Patrick, & Dani, 1992), which oppose the depolarization caused by nAChR opening. nAChRs differentially modulate neuronal excitability, depending on the target cell, and the strength and timing of the cholinergic input (Frazier et al., 1998; Ji & Dani, 2000; Alkondon & Albuquerque, 2004).

Intrahippocampal administration of the nAChR agonist nicotine facilitates working memory (Felix & Levin, 1997; Levin, McClernon, & Rezvani, 2006), while intrahippocampal administration of the nAChR antagonists dihydro-b-erythroidine, methyllycaconitine (Felix & Levin, 1997; Levin, Bradley, Addy, & Sigurani, 2002) or mecamylamine (Ohno, Yamamoto, & Watanabe, 1993) impairs working memory. Nicotinic receptors in the CA1 region of the hippocampus have been involved in both STM and LTM formation, and in retrieval processes of an IA response in rats, suggesting that nAChRs have a modulatory role in different types and phases of memory (Marti, Ramirez, Dos Reis, & Izquierdo, 2004). Systemic nicotine administration 15 min prior to a retrieval test ameliorates IA memory. This effect is opposed by the centrally acting antagonist mecamylamine but not by the peripherally acting antagonist

hexamethonium or the muscarinic antagonist atropine all given IP (Zarrindast, Sadegh, & Shafaghi, 1996). Post-training intracerebroventricular infusions of ACh or nicotine have been shown to enhance inhibitory avoidance. This effect is reduced by coinfusion of scopolamine (Eidi, Zarrindast, Eidi, Oryan, & Parivar, 2003).

Using microdialysis in freely moving rats, it was shown that hippocampal memory processes are associated with a marked increase in ACh release (Ragozzino, Pal, Unick, Stefani, & Gold, 1998; Stancampiano, Cocco, Cugusi, Sarais, & Fadda, 1999; Giovannini et al., 2001b; Giovannini et al., 2005; Bianchi et al., 2003). Behavioural conditions that induce arousal, require attention and lead to information acquisition and memory formation, are associated and supported by activation of the forebrain cholinergic system (Demeter & Sarter, 2013).

Disruption of the hippocampal cholinergic input in animals further demonstrates the importance of this structure in cognitive processes. Inhibition of ACh synthesis induced by hemicholinium-3 ICV administration, a selective inhibitor of high-affinity choline uptake (Gardiner, 1961) leads to memory consolidation impairment in the IA task in mice (Boccia, Acosta, Blake, & Baratti, 2004). Power & McGaugh (2002), using the nonselective cholinergic excitotoxin phthalic acid injected in the NBM, found that phthalic acid-lesioned animals showed a significant reduction of inhibitory avoidance learning. This impairment could be rescued by ipsilateral infusions of the muscarinic agonist oxotremorine or the acetylcholinesterase inhibitor physostigmine. Furthermore, intra-hippocampal injection of the muscarinic receptor antagonist scopolamine impairs memory acquisition in a IA task (Giovannini et al., 2005) and spatial discrimination learning in the Morris water maze (Blokland, Honig, & Raaijmakers, 1992). IP administration of atropine, a central muscarinic antagonist, completely prevents the facilitatory effects of the central β 2-adrenoreceptor agonist, clenbuterol also given IP (Introini-Collison & Baratti, 1992), suggesting an interaction between central adrenergic and cholinergic mechanisms in the IA response in mice. Furthermore, selective lesion of the medial septal and diagonal band cholinergic neurons resulted in deficits in spatial strategies used to locate a spatial goal in the Morris water maze (Janis, Glasier, Fulop, & Stein, 1998). Lesions of the cholinergic and/or GABAergic neurons in the medial septum and diagonal band showed that GABAergic and cholinergic septohippocampal neurons both contribute to memory stabilization (Lecourtier et al., 2011) whereby GABAergic processes could be engaged at an earlier stage than cholinergic ones during system consolidation of a spatial memory. Disruption of the GABAergic neurons of the medial septum and diagonal band impairs ACh efflux and

working memory under the heavy memory load of a delayed non matching to position task, but does not alter hippocampal ACh efflux and easier memory tasks (Roland et al., 2014).

The toxin 192 IgG-saporin at present is the most convenient tool to induce selective cholinergic denervation (Waite & Thal, 1996; Wiley, Oelmann & Lappi 1991). 192 IgG-saporin is constituted of the monoclonal antibody 192 IgG which has a low affinity to nerve growth factor (NGF) receptor p75 present on cholinergic neurons and saporin, a ribosome inactivating toxin. The 192 IgG-saporin binds to the p75 NGF receptors, is internalized and retrogradely transported to the soma, where it is cleaved. Saporin disrupts the ribosomal function, thus leading to cell death (Wiley, Oelmann & Lappi 1991).

The intracerebral injection of this toxin to disrupt cholinergic neurons has given controversial results. Although 192 IgG-saporin brings about selective and significant cholinergic damage of the NBM, only modest deficits in mnemonic tasks have been reported (Torres et al., 1994; Baxter et al., 1996). For instance, 192 IgG-saporin lesions had no effect on inhibitory avoidance learning (Power, Thal & McGaugh, 2002). Some of the authors explained the unexpectedly modest effect of immunotoxin lesions on memory paradigms with the possible existence of compensatory mechanisms after the lesions (Lacroix, White & Feldon, 2002; de Bruin, Ellenbroek, van Luijtelaa, Cools & Stevens, 2001), or with the immunotoxin selective effect on cortical projections and comparative lack of effect on amygdalopetal cholinergic projections (Power, Thal & McGaugh, 2002). More recently it has been shown that only ICV lesions, but not NBM lesions using 192 IgG-saporin lead to memory impairments in passive avoidance and Morris water maze tasks (Garcia-Alloza et al, 2006). Also, rats with immunotoxic lesions of cholinergic neurons in the MS/VDB, are unimpaired in a test of “episodic-like” memory (Easton, Fitchett, Eacott & Baxter, 2011). On the other hand, very recently it has been demonstrated that 192 IgG-saporin impairs spatial learning (Rastogi, Unni, Sharma, Rao Laxmi & Kutty, 2014). Others demonstrated that 192 IgG-saporin causes basal forebrain cholinergic depletion and impairs working memory, spatial discrimination, social novelty preference (Cutuli et al., 2013), and these effects are prevented by administration of donepezil, an indirect cholinomimetic drug. The different types of behavioural tests used and memories studied as well as the participation of other neurotransmitter systems in learning and memory mechanisms may explain the contrasting effects of 192 IgG-saporin lesions described in the literature.

3. The cholinergic system and ERK transduction pathway in memory formation

ACh, released in the proximity of depolarized neurons, brings about a long-lasting potentiation of the postsynaptic membrane (Tremblay, Warren, & Dykes, 1990; Metherate, 1998). Stimulation of muscarinic/nicotinic receptors subtypes present on neurons (Rosenblum, Futter, Jones, Hulme, & Bliss, 2000; Dineley et al., 2001; Berkeley et al., 2001; Giovannini et al., 2008) may create conditions favourable for neuronal plasticity, initiating a network of signals that activate several intracellular transduction pathways including the ERK pathway (Gutkind, 1998). ERK is also activated by glutamate through metabotropic (Peavy & Conn, 1998) or ionotropic glutamate receptors (Zhu, Qin, Zhao, Van Aelst, & Malinow, 2002; Krapivinsky et al., 2003), by noradrenaline through β -adrenergic receptors (Williams, Zhong, & Minneman, 1998; Winder et al., 1999; Watabe, Zaki, & O'Dell, 2000), by other neurotransmitters (Drutel et al., 2001; Giovannini et al., 2003), and growth factors (Castillo & Escobar, 2011) and by the sex steroid hormones 17 β -estradiol and progesterone (Harburger, Saadi, & Frick, 2009; Orr, Rubin, Fan, Kent, & Frick, 2012).

It has been demonstrated that combined stimulation of mAChR and β -adrenergic receptors synergistically activate ERK which can act as a coincidence detector (to decode the simultaneous engagement of different receptors) and as a signal integrator (that encodes this information in a spatially and temporally distinct biological signals) (Watabe, Zaki, & O'Dell, 2000; Sweatt, 2001; Geetha et al., 2011), thus activating a cascade of intracellular processes that lead to synaptic plasticity and learning. Indeed, ERKs are placed at a strategic position allowing crosstalk between different arrays of signals and signal transduction pathways.

ERK is localized in the soma and dendritic trees of neurons in the neocortex, hippocampus, striatum, and cerebellum (Fiore et al., 1993). Phosphorylation of ERK by its upstream kinase MEK is necessary for the formation of different types of learning and memory (Atkins, Selcher, Petraitis, Trzaskos, & Sweatt, 1998; Blum, Moore, Adams, & Dash, 1999; Kaminska, Kaczmarek, Zangenehpour, & Chaudhuri, 1999; Walz et al., 2000; Cammarota et al., 2000). The first direct evidence that ERK is involved in memory processes *in vivo* was reported in a seminal paper published by Sweatt's group (Atkins, Selcher, Petraitis, Trzaskos, & Sweatt, 1998). These findings (Atkins, Selcher, Petraitis, Trzaskos, & Sweatt, 1998) were confirmed and expanded using inhibitors of ERK activation in the rat (PD098059 or U0126 injected intracerebrally) (Schafe, Nadel, Sullivan, Harris, & LeDoux, 1999; Schafe et al., 2000). Later studies showed that activation of the ERK hippocampal pathway is required for long-term fear memory (Giovannini et al., 2003; Apergis-Schoute, Debiec, Doyere, LeDoux, & Schafe, 2005).

Downstream effectors of ERK activation

Within minutes from activation, a fraction of phospho-ERK translocates to the nucleus (Davis, Vanhoutte, Pages, Caboche, & Laroche, 2000), where it can modify gene expression by transcriptional control (Xia, Dudek, Miranti, & Greenberg, 1996; Impey et al., 1998). It is likely that ERK participates in different forms of neuronal plasticity by virtue of its ability to regulate both transcription at the nuclear level (for a review, see Impey et al., 1998; Chang & Karin, 2001) and translation in the dendrites (Chen, Rojas-Soto, Oguni, & Kennedy, 1998; Kim, Liao, Lau, & Huganir, 1998; Flood et al., 1998). The former effect is consistent with the participation of ERK in memory formation through protein synthesis (Chwang, Arthur, Schumacher, & Sweatt, 2007) whereas the translational effects occur through phosphorylation and changes in local synaptic mechanisms (English & Sweatt, 1996; English & Sweatt, 1997; Impey et al., 1998; Giovannini et al., 2001a). Thus, it seems plausible that ERK participates in both forms of memory, by modifying the existing proteins that determine synaptic behavior, and/or by regulating the expression of proteins necessary for the long-term maintenance of synaptic changes. Some of these latter effects are thought to reflect ERK-dependent activation of transcription factors such as CREB and Elk-1 (Treisman, 1995; Treisman, 1996; for review see Sweatt, 2001).

After activation, the fraction of ERK that remains in the dendrites is extensively phosphorylated (Impey et al., 1998; Winder et al., 1999; Giovannini et al., 2005) pointing to an involvement of ERK in the activation of downstream cytoplasmic proteins such as mTOR (Tsokas, Ma, Iyengar, Landau, & Blitzer, 2007), ribosomal S6 kinase2, RSK2 (Poteet-Smith, Smith, Lannigan, Freed, & Sturgill, 1999), that regulate translational efficiency (Grewal, York, & Stork, 1999). Other extranuclear substrates for ERK include components of the postsynaptic signalling network such as phospholipase A₂ (Xu et al., 2002), SynGAP (Muthalif, Benter, Uddin, & Malik, 1996; Chen, Rojas-Soto, Oguni, & Kennedy, 1998; Kim, Liao, Lau, & Huganir, 1998), and several microtubule-associated proteins (MAP), such as MAP-1, MAP-2, MAP-4, and Tau (Seger & Krebs, 1995). Furthermore, the postsynaptic density, a subsynaptic complex in which much of the postsynaptic signalling occurs, includes ERK2, MEK, and the phosphatase (MKP2) (Husi, Ward, Choudhary, Blackstock, & Grant, 2000). Furthermore, dendritic phospho-ERK appears to play an important role in regulating K⁺ channels, particularly in the phosphorylation of the pore-forming α subunit of Kv4.2 channels. Likely, this role contributes to dendritic information processing and increasing membrane excitability (Yuan, Adams, Swank, Sweatt, & Johnston, 2002; Watanabe, Hoffman, Migliore, & Johnston, 2002; Morozov et al., 2003; Sweatt, 2004).

Adding an even higher level of complexity to the involvement of ERK in memory mechanisms, it has been shown that the ERK cascade is involved in epigenetic mechanisms (Berger, Kouzarides, Shiekhata, & Shilatifard, 2009) in the hippocampus, such as downstream histone H3 acetylation and phosphorylation *via* nuclear kinases (Levenson et al., 2004; Chwang, O'Riordan, Levenson, & Sweatt, 2006; Chwang, Arthur, Schumacher, & Sweatt, 2007). Stimulation of ERK signalling (Levenson et al., 2004) produces gene- and histone-specific changes in post translational modifications, indicating that distinct signalling cascades may establish precise histone codes that correspond to particular types of memory (Graff, Kim, Dobbin, & Tsai, 2011). Together, these findings support the possibility that ERK may play a role in memory both through nuclear and local synaptic mechanisms dependently and/or independently on gene transcription.

Role of ERK1 and ERK2

Two isoforms of ERK are present in cells, ERK1 (p44MAPK) and ERK2 (p42MAPK), which have similar distribution in the brain, although the amount of ERK1 in neurons of rat hippocampus appears to be considerably lower than that of ERK2 (Kanterewicz et al., 2000; Giovannini et al., 2001a). The two isoforms share about 90% homology (Boulton et al., 1990) and have the same substrate specificity *in vitro*, but their role *in vivo* remains to be elucidated. It is still not fully understood whether both isoforms are equally involved in learning and memory mechanisms. Several groups have found that in neurons ERK1 and ERK2 are selectively regulated by different stimuli (Bading & Greenberg, 1991; Fiore, Murphy, Sanghera, Pelech, & Baraban, 1993; English & Sweatt, 1996; Giovannini et al., 2001a), and it has been suggested that only ERK2 plays a key role in synaptic plasticity and memory consolidation (Sweatt, 2001). Knockout (KO) mice for ERK1 and ERK2 have been generated and, whereas ERK1 KO mice are viable and appear to be neurologically normal (Selcher, Nekrasova, Paylor, Landreth, & Sweatt, 2001), ERK2 KO mice are embryonic lethal at day 6.5 (Yao et al., 2003; Saba-El-Leil et al., 2003). Therefore the two isoforms must have some important different function, at least early in mouse embryonic development (Saba-El-Leil et al., 2003). Selcher and coworkers demonstrated in KO mice that ERK1 is not required for emotional learning whereas ERK2 has a predominant role in synaptic plasticity underlying learning and memory processes (Selcher, Nekrasova, Paylor, Landreth, & Sweatt, 2001). It has also been shown (Mazzucchelli et al., 2002) that in ERK1 KO mice, STM is retained but there is a marked enhancement of LTM in a one-trial IA task. The view that the ERK2 isoform exerts a pivotal role in LTM modulation is supported also by the results of a

reconsolidation study (Cestari, Costanzi, Castellano, & Rossi-Arnaud, 2006) in which administration of SL327, an inhibitor of ERK activation, impaired memory reconsolidation not only in wt mice, but also in ERK1 KO mice. Altogether, these results clearly show a central role for ERK2 activation in memory reconsolidation processes in mice (Cestari, Costanzi, Castellano, & Rossi-Arnaud, 2006). It has also been suggested that ERK1 has a physiological inhibitory role on MEK (Mazzucchelli et al., 2002), thus limiting ERK1/2 activation. Some possible explanations for the selective activation of ERK2 in learning and memory mechanisms are its specific activation by upstream kinases, compartmentalization, differences in the brain structures involved, and binding to scaffolding proteins through highly specific docking sites (Sharrocks, Yang, & Galanis, 2000; Enslin & Davis, 2001), but so far there is no compelling evidence for any of these.

ERK activation in IA memory

As already mentioned, activation of the basal forebrain cholinergic pathway during memory acquisition, and the subsequent release of ACh, leads to stimulation of mAChRs that in turn trigger ERK activation either via PKC (Yasoshima & Yamamoto, 1997) or PYK2 (Lev et al., 1995). More recently, it has been demonstrated that nicotine may enhance hippocampus-dependent learning, most likely by impinging on $\alpha 4\beta 2$ nAChRs and activating intracellular PKA and ERK pathways. Indeed, administration of the PKA inhibitor PKI 14-22 amide in the dorsal hippocampus (Gould et al., 2014) or an ERK inhibitor (SL327, administered IP) (Raybuck & Gould, 2007), at doses too low to impair learning *per se*, blocks learning facilitation elicited by nicotine. This suggests that nicotine administration during learning increases PKA and ERK activation and, if this increase is blocked, learning is impaired (Gould & Leach, 2014). Nicotine administered IP prior to learning increases CREB phosphorylation at the Jnk1 promoter region (Kenney, Poole, Adoff, Logue, & Gould, 2012) and Jnk1 expression in the hippocampus (Kenney, Florian, Portugal, Abel, & Gould, 2010). Thus, the ability of nicotine to modify cell signalling cascades involved in learning and to express additional signalling cascades may explain why nicotine administration is associated with the formation of a strong drug-context memory contributing to the drug seeking behaviour (Walters, Cleck, Kuo, & Blendy, 2005; Wilkinson & Bevins, 2008; Portugal & Gould, 2009; Gould & Leach, 2014).

Few investigations exist which directly correlate the increase in ACh release to performance of an IA task in the rat. We demonstrated (Giovannini et al., 2005) that IA acquisition initiates a cascade of events that activates hippocampally-projecting cholinergic

neurons. This is revealed by an increase in ACh release during and immediately after acquisition (Giovannini et al., 2005), by the activation of ERK in CA1 hippocampal neurons and memory formation. We showed (Giovannini et al., 2005) that administration of the mAChRs antagonist scopolamine (IP, or locally into the hippocampus) prior to training, and of ERK inhibitors U0126 and PD98059 (locally into the hippocampus), cause both inhibition of ERK activation and amnesia, demonstrating that both ACh release and ERK activation are necessary for IA STM formation. These results indicate that an increase in ACh release acting on postsynaptic mAChRs triggers an intracellular signalling cascade that activates ERK and further downstream effectors leading to memory encoding.

A time-window for ERK activation to be efficacious must exist, since it has been demonstrated that, at least in spatial memory, delayed infusion of MEK inhibitors U0126 and PD98059 does not interfere with long term spatial memory retention (Blum, Moore, Adams, & Dash, 1999). Since the neuronal ERK cascade can function as a biochemical coincidence detector, being activated simultaneously by β -adrenergic receptors and mAChRs in the hippocampus (Watabe, Zaki, & O'Dell, 2000), it is feasible that neurotransmitter systems other than the cholinergic may be concomitantly activated during acquisition. It is also possible that blocking one of the converging pathways is sufficient to inhibit the entire ERK cascade and the encoding of the IA memory. These findings show that an aversive experience such as the exposure to a single footshock initiates a cascade of events that, through the activation of hippocampally-projecting cholinergic neurons, promotes the activation of the ERK pathway and leads to the formation of STM of that event.

Izquierdo's group (Cammarota et al., 2000) demonstrated that learning of IA is associated with a similar, NMDA dependent, activation of both ERK1 and ERK2 in the rat hippocampus. In a further series of papers (Walz et al., 1999; Walz et al., 2000) the authors found that inhibition of ERK activation by PD 098059 in the CA1 region, entorhinal cortex, parietal cortex or amygdala impaired retention of the IA when tested up to 6 h after training, with a differential time course in the different brain regions. The authors thus concluded that the ERK pathway is involved in the IA post-training memory consolidation, with a different time-course in the hippocampus, amygdala, entorhinal cortex or parietal cortex of rats (Walz et al., 2000). It seems that activation of ERK correlates mostly with the aversive, emotional, component of IA acquisition since it was previously found that ERK is activated by mild electric footshocks similar in intensity and length to that employed as an aversive stimulus during IA training (Bevilaqua et al., 2006), but not by exposure to the training box in the absence of the footshock (Alonso, Viola, Izquierdo, & Medina, 2002). More recently,

experiments from the same group (Igaz, Bekinschtein, Izquierdo, & Medina, 2004) demonstrated that single trial step-down IA causes an increase of total ERK mRNA 3 h, and ERK2 protein upregulation 24 h after training. These results are interesting in that they reveal that ERK activity can possibly be modulated in different behavioural tasks not only by activation, but also by increased protein expression.

ERK activation in STM and LTM formation

Although some authors showed that ERK activation participates in LTM but not STM (Atkins, Selcher, Petraitis, Trzaskos, & Sweatt, 1998; Berman, Hazvi, Rosenblum, Seger, & Dudai, 1998; Blum, Moore, Adams, & Dash, 1999), data from our and other laboratories indicate that a rapid and transient activation of ERK participates in the molecular mechanisms underlying IA STM formation (Giovannini et al., 2005; Walz et al., 1999; Izquierdo et al., 2000; Alonso, Viola, Izquierdo, & Medina, 2002). The effects of inhibitors of ERK activation injected immediately post training into CA1 or entorhinal cortex on STM and LTM show an interesting mirror image. In CA1, the role of ERK appears to be restricted mainly to STM formation. Simultaneously, in the entorhinal cortex the activation of this pathway impairs STM formation but is necessary for LTM formation. Studies with the MEK inhibitor PD98059, injected intracortically at different times after training, point to a role of ERK in LTM consolidation (Walz et al., 1999; Walz et al., 2000). These data suggest that the ERK pathway plays a complex regulatory role in synaptic plasticity. It is linked at different levels with the PKC, CaMKII, and PKA cascades (Bhalla & Iyengar, 1999; Lisman & Fallon, 1999) which are all crucial for LTM and, depending on brain structure and post training time, also for STM (Cammarota, Bevilacqua, Medina, & Izquierdo, 2007).

In agreement with the literature (Walz et al., 1999; Walz et al., 2000; Alonso, Viola, Izquierdo, & Medina, 2002), the duration of ERK activation is limited to a restricted time window after training, indicating that acquisition of aversive experiences is associated with a rapid and short lasting activation of ERK. According to these data, it seems that an inherent signal termination process limits the duration of ERK activation (Pouyssegur, Volmat, & Lenormand, 2002), which depends upon a balance between the activity of kinases and phosphatases (Kwak, Hakes, Martell, & Dixon, 1994; Misra-Press, Rim, Yao, Roberson, & Stork, 1995; Muda et al., 1996; Boschert, Dickinson, Muda, Camps, & Arkininstall, 1998). Many of these latter proteins are induced by stimuli that also activate ERK and participate in a negative feedback control of ERK activity (Paul, Nairn, Wang, & Lombroso, 2003) in which ERK itself determines the duration of its own activation by in turn activating phosphatases

(Pouyssegur, Volmat, & Lenormand, 2002). Since ERK can function as a biochemical signal integrator in response to extracellular signals in neurons (Watabe, Zaki, & O'Dell, 2000), the ramifications of ERK-dependent signalling are determined, in part, by the duration of ERK phosphorylation itself and it may not be surprising to find that the duration of its activation is tightly regulated. Indeed, short-term activation of ERK triggers cell differentiation in PC12 cells, while prolonged activation results in cell proliferation (Traverse, Gomez, Paterson, Marshall, & Cohen, 1992). Repeated depolarizations, rather than a single depolarization, cause sustained ERK activation, which is essential for new spine formation in neuron culture, since MEK inhibition with PD98059 prevents both ERK activation and spine formation events (Wu, Deisseroth, & Tsien, 2001).

Baseline ERK activation decreases in basal conditions in the hippocampus of TgCRND8 mice, an early-onset transgenic mouse model of Alzheimer's Disease (AD) (Chishti et al., 2001; Bellucci et al., 2006). Cholinergic stimulation *ex vivo* strongly increases ERK activation in the cell bodies of CA1 pyramidal neurons and of DG granule cells of wild type (wt) mice, showing that activation of ERK in these neurons is downstream of cholinergic activation (Bellucci et al., 2006). This effect is significantly smaller in the hippocampus of transgenic mice, indicating a possible mechanism responsible for the memory deficits present in TgCRND8 mice (Bellucci et al., 2006). Most interestingly, the cholinergic agonist carbachol induced a much lower activation of ERK in TgCRND8 mice hippocampal slices *in vitro* than in slices from wt littermates (Giovannini et al., 2008). This effect may be ascribed to modifications upstream of ERK, such as a decrease in the number of mAChRs which are significantly reduced in TgCRND8 mice (Bellucci et al., 2006). Thus, these findings offer a molecular basis for memory disruption in AD, since memory requires proper functioning of the basal forebrain cholinergic neurons (Sarter & Bruno, 1997b), and ERK2 activation (Atkins, Selcher, Petraitis, Trzaskos, & Sweatt, 1998; Blum, Moore, Adams, & Dash, 1999; Kaminska, Kaczmarek, Zangenehpour, & Chaudhuri, 1999; Walz et al., 2000; Cammarota et al., 2000; Adams & Sweatt, 2002):

As mentioned above, it appears (Pouyssegur, Volmat, & Lenormand, 2002) that the kinetics and duration of ERK activation may play an important role in influencing its effect on cell fate. Obviously, differential durations of ERK activation are regulated by different molecular players (York et al., 1998; Corbit, Foster, & Rosner, 1999) and can elicit unique gene expression profiles, and/or protein translation which consequently result in different cellular functions (Marshall, 1995) and final cellular outcomes. The possibility that sustained ERK can activate death programs independent of caspases in neurons (Subramaniam et al.,

2004) suggests that ERK activation is involved in non-apoptotic modes of neuronal death. On the contrary, activation of ERK and Akt has been found to confer neuroprotection in several models of apoptosis (Hetman, Kanning, Cavanaugh, & Xia, 1999). Similarly, both Akt and ERK have been reported to play a role in regulating hippocampal neurogenesis (Aberg et al., 2003).

4. The Cholinergic system and mTOR pathway in memory formation

LTM formation require protein synthesis, and direct translation in the synapto-dendritic compartment represents a mechanism that can produce rapid changes in protein content in response to synaptic activity (Bailey, Kandel, & Si, 2004; Kelleher, III, Govindarajan, Jung, Kang, & Tonegawa, 2004; Hoeffler & Klann, 2010).

The Mammalian Target of Rapamycin (mTOR), is an evolutionary conserved high molecular weight serine-threonine protein kinase that regulates cell growth, proliferation and survival (Martin & Hall, 2005) by increasing protein translation. In the brain, mTOR regulates protein translation in response to neuronal activity, thereby modulating synaptic plasticity and LTM formation (Kelleher, III, Govindarajan, Jung, Kang, & Tonegawa, 2004). Downstream targets of mTOR include p70S6K, and eukaryotic Elongation Factor 1A and 2 (eEF1A and eEF2), which are mostly involved in ribosome recruitment to mRNA, the eukaryotic initiation factor 4E binding proteins (4E-BPs), which regulate both the initiation and elongation phases of translation (Hay & Sonenberg, 2004).

Activation of mTOR by growth factors is well documented (Hay & Sonenberg, 2004; Slipczuk et al., 2009). It has also been demonstrated that mTOR can be activated by GPCRs (Wang & Proud, 2002; Arvisais, Romanelli, Hou, & Davis, 2006) and in particular it was demonstrated in a neuroblastoma cell line *in vitro* that the mTOR pathway is activated by mAChRs (Slack & Blusztajn, 2008). Furthermore, mTOR activation is downstream of nAChRs in cultured non-small-cell lung carcinoma cells (Zheng, Ritzenthaler, Roman, & Han, 2007). The above results indicate that mTOR can be downstream of cholinergic receptors.

The mTOR pathway was first implicated in synaptic plasticity when it was shown that rapamycin, a selective inhibitor of mTOR Complex 1 (mTORC1) activity (Casadio et al., 1999; Takei, Kawamura, Hara, Yonezawa, & Nawa, 2001; Hay & Sonenberg, 2004; Takei et al., 2004) blocks long-term facilitation in *Aplysia* (Casadio et al., 1999) In addition, mTOR-dependent activation of dendritic ribosomal protein kinase (p70S6K) was shown to be

necessary for the induction phase of protein-synthesis-dependent synaptic plasticity (Cammalleri et al., 2003).

Whereas several studies have examined the effects of mTOR inhibition on synaptic plasticity *in vitro* (Tsokas, Ma, Iyengar, Landau, & Blitzer, 2007), few have examined the role of mTOR in learning and memory *in vivo* (Parsons, Gafford, & Helmstetter, 2006; Bekinschtein et al., 2007). The latter authors were the first to demonstrate that acquisition or consolidation of fear memories in the hippocampus or amygdala require mTOR activity (Parsons, Gafford, & Helmstetter, 2006; Bekinschtein et al., 2007). Furthermore, central administration of rapamycin *in vivo* disrupts the formation of different types of memories (Tischmeyer et al., 2003; Parsons, Gafford, & Helmstetter, 2006; Dash, Orsi, & Moore, 2006; Schicknick et al., 2008; Sui, Wang, & Li, 2008; Belelovsky, Kaphzan, Elkobi, & Rosenblum, 2009).

Interestingly, it was reported that in cultured neurons and hippocampal slices from AD transgenic mice and in hippocampal slices from wt mice, exposed to exogenous A β 1-42, the mTOR signalling pathway is inhibited (Ma et al., 2010). The mTOR dysregulation correlates with impairment in synaptic plasticity. On the contrary, upregulation of mTOR signalling by both pharmacological and genetic methods prevents A β -induced synaptic impairment, indicating that dysregulation of the mTOR pathway could play a role in the synaptic dysfunction that characterizes AD (Ma et al., 2010). As mentioned above, the effects of ACh on learning and memory in the hippocampus appear to be mediated mainly by mAChRs (Izquierdo et al., 1998b; Barros, Pereira, Medina, & Izquierdo, 2002; Giovannini et al., 2005), although there is evidence indicating that nAChRs have an important modulatory role (Decker, Brioni, Bannon, & Arneric, 1995; Marti, Ramirez, Dos Reis, & Izquierdo, 2004; Mitsushima, Sano, & Takahashi, 2013). Some years ago, it was demonstrated (Feig & Lipton, 1993) that activation of mAChRs stimulates new protein synthesis in hippocampal CA1 dendrites. Since then little progress has been made in understanding the role of local protein synthesis in mAChR-dependent synaptic plasticity. Recent results that demonstrated that the mTOR pathway is downstream of mAChRs and nAChRs (Zheng, Ritzenthaler, Roman, & Han, 2007; Slack & Blusztajn, 2008), link the cholinergic system to mTOR activation and to local protein synthesis via multiple MAPK- and/or PKC-dependent mechanisms. Specifically, M2 receptors utilize a MAPK-dependent mechanism to activate this pathway, whereas M3 receptors utilize either MAPK dependent or independent mechanisms, depending on cellular context (Slack & Blusztajn, 2008). Furthermore, in a

recent paper the authors demonstrate a fine regulation of mTOR and p70S6K by the muscarinic M4 receptor in PC12 cells (Chan, Wu, & Wong, 2009).

In a recent study from our laboratory, rapamycin (injected ICV) was used as a pharmacological tool to dissect the intracellular translational machinery activated by upstream signals in the acquisition and retrieval of an IA memory (Lana et al., 2013). We showed that mTOR and its downstream effector p70S6K are massively activated in most of CA1 pyramidal neurons at early times after acquisition of an IA memory (Lana et al., 2013). A fairly rapid and transient inactivation of mTORC1 and, consequently, of p70S6K by rapamycin impairs formation of LTM with no effect on STM, demonstrating that mTORC1 activation is necessary for LTM. These data are consistent with those reported by Hoeffler who demonstrated that rapamycin disrupts fear associated LTM formation 24 h, but not 3 h, after acquisition (Hoeffler et al., 2008).

An intriguing result (Lana et al., 2013) is the observation that 1 h after administration of the mAChR antagonist scopolamine, activation of mTOR and p70S6K is increased. Presynaptic M2/M4 mAChRs, located on septo-hippocampal cholinergic terminals (Quirion et al., 1995), act as inhibitory autoreceptors (Raiteri, Lardi, & Marchi, 1984; Douglas, Baghdoyan, & Lydic, 2002; Zhang et al., 2002) and their blockade by scopolamine (Figure 1) massively increases ACh release (Scali, Vannucchi, Pepeu, & Casamenti, 1995), which, in the presence of the non-selective muscarinic antagonist scopolamine, only binds to postsynaptic nAChRs leading to activation of the mTOR pathway. The link of nAChR to the mTOR pathway has been demonstrated in other systems (Zheng, Ritzenthaler, Roman, & Han, 2007; Sun et al., 2009) and, as reported above, the involvement of nAChR to mediate ACh postsynaptic responses in the hippocampus is substantiated by several studies (Marti, Ramirez, Dos Reis, & Izquierdo, 2004; Bell, Shim, Chen, & McQuiston, 2011; Gu & Yakel, 2011). An alternative explanation of the increase in mTOR activation following scopolamine may be that the large and long-lasting increase of ACh release evoked by scopolamine (Scali, Vannucchi, Pepeu, & Casamenti, 1995) may in time overcome the postsynaptic antagonistic effect of the muscarinic competitive antagonist, with the ensuing activation of intracellular pathways and consequent increase of the mTOR intracellular signalling. This may trigger protein translation, presumably responsible for LTM formation (Bekinschtein et al., 2010). In a recent study (Mitsushima, Sano, & Takahashi, 2013), it was found that ACh mediates learning-induced strengthening at excitatory and inhibitory synapses through distinct sets of AChRs. Activation of mAChRs mediates the IA learning through the incorporation of AMPA-type glutamate receptors into hippocampal CA3-CA1 synapses. IA learning also

strengthens inhibitory hippocampal synapses through the activation of nAChRs but not mAChRs. These data reveal novel molecular and cellular mechanisms of learning-dependent synaptic plasticity. Thus, ACh balances the excitatory and inhibitory synaptic inputs onto CA1 pyramidal neurons in IA learning through the activation of distinct sets of AChRs (Mitsushima, Sano, & Takahashi, 2013). Therefore, ACh function on any given circuit and intracellular pathways may depend on the specific expression of postsynaptic mAChRs versus nAChRs and upon the temporal dynamics of ACh levels in the synaptic cleft.

In Figure 1 the contribution of the cholinergic system and the downstream effectors ERK and mTOR in the formation of inhibitory avoidance (IA) short term and long term memories (STM, LTM) is shown. ACh released by presynaptic terminal activates both muscarinic and nicotinic postsynaptic receptors (mAChRs, nAChRs). Postsynaptic mAChRs and nAChRs indirectly activate the intracellular pathways of ERK and mTOR, responsible, with different contribution, and different downstream effectors, for IA STM and LTM formation. Inhibitory mAChRs presynaptic receptors, blocked by muscarinic antagonists scopolamine and atropine, lead to massive increase of ACh release.

As shown in Figure 1, administration of muscarinic plus nicotinic antagonists *in vivo* blocks the scopolamine-induced increase of mTOR activation 1 h after administration (Lana et al., 2013). However, although mTOR is activated only at a later time (4 h) after administration of the drugs, LTM is still maintained (Lana et al., 2013). It is therefore possible that activation of mTOR at later times is sufficient to activate downstream effectors leading to LTM formation. The apparent discrepancy between the effect of muscarinic plus nicotinic antagonists on LTM formation and the decreased activation of mTOR and p70S6K may also be explained considering that several other neurotransmitter systems (Izquierdo et al., 1998c; Slipczuk et al., 2009) and other intracellular signalling pathways are involved in IA LTM formation (Khakpai, Nasehi, Haeri-Rohani, Eidi, & Zarrindast, 2012). A further explanation for this apparent discrepancy may come from data that demonstrate that in some instances mTORC1 activity regulates only a small component of total protein synthesis (Yanow, Manseau, Hislop, Castellucci, & Sossin, 1998; Choo, Yoon, Kim, Roux, & Blenis, 2008) and additional mTORC1-independent regulatory signals are required to induce memory since stimulation of mTORC1 probably generates a set of proteins important, but not sufficient for neuronal plasticity or memory (Graber, McCamphill, & Sossin, 2013).

A further demonstration that mTOR is downstream of mAChR activation derives from *in vitro* experiments on rat hippocampal slices. Carbachol significantly increases mTOR and p70S6K activation in CA1 pyramidal neurons *in vitro*. This effect is antagonized by the

mAChRs antagonist scopolamine (IP) and the nAChRs antagonist mecamylamine (ICV) administered together before carbachol (Lana et al., 2013).

Although at variance from data reported by some investigators (Marti, Ramirez, Dos Reis, & Izquierdo, 2004), these results (Lana et al., 2013) support the idea that scopolamine predominantly affects STM processes (Givens & Olton, 1995; Stanhope, McLenachan, & Dourish, 1995; Savage, Faust, Lambert, & Moerschbaecher, 1996; Estape & Steckler, 2002). These data are in accordance with data demonstrating that blockade of mAChRs by scopolamine given IP to animals is followed by an impairment of working memory and the disruption of recently acquired tasks, resembling the impairment of recent memory in humans, with no effect on spatial reference memory (Bartolini, Risaliti, & Pepeu, 1992) or maze performance in overtrained animals (Pazzagli & Pepeu, 1964).

A further refinement of the effect of mTOR activation on protein translation may come from the effects of downstream effectors of mTORC1, such as 4E-BP1, on protein translation. These effects are not limited simply to switching ‘off’ or ‘on’ protein synthesis; they can also alter the range and the type of nascent proteins by mediating a switch between cap-dependent and cap-independent translation (Bove, Martinez-Vicente, & Vila, 2011).

Finally, recent reports indicate that LTM deficits can be associated with hyperactivation of the mTOR signalling pathway and an imbalance in protein synthesis (Bolduc, Bell, Cox, Brodie, & Tully, 2008). Puighermanal et al (2009) demonstrated that activation of the Cannabinoid receptor type 1 in the mouse hippocampus *in vivo* modulates the mTOR pathway, activating p70S6K and increasing protein translation. Contrary to what may be expected, in this case, an increase in protein translation seems to be responsible for the memory impairments caused by cannabinoid consumption (Puighermanal et al., 2009). In the same direction lead the findings showing that, although basal mTOR activity seems to be necessary for memory consolidation, an increase in mTOR signalling can disrupt memory processing. In patients with tuberous sclerosis and in animal models of this genetic disease, mutations that cause a reduction in Tuberous Sclerosis Complex1–Tuberous Sclerosis Complex2 activation and an increase in mTORC1 activity are associated with memory deficits (Ehninger et al., 2008).

Interplay between ACh and the ERK and mTOR pathways in IA memory encoding

A mechanistic model that may help explaining the integrated role of cholinergic activation and the downstream effectors ERK and mTOR in the formation of hippocampal inhibitory avoidance short term and long term memories is shown in Figure 1. The

cholinergic septo-hippocampal pathway is activated during acquisition of an IA memory (Giovannini et al., 2005) and ACh, released by presynaptic terminals, impinges on and activates both muscarinic and nicotinic postsynaptic receptors. Postsynaptic mAChRs indirectly activate the intracellular pathways of ERK and mTOR (dotted arrows), responsible, with different contributions and different downstream effectors, for IA STM and LTM formation (Giovannini et al., 2005; Lana et al., 2013). Inhibitory muscarinic presynaptic receptors, blocked by muscarinic antagonists, lead to massive increase of ACh release (Scali et al., 1995) that impinges on postsynaptic nAChRs, not blocked by muscarinic antagonists. Activation of postsynaptic nAChRs indirectly leads to activation of mTOR and formation of the mTORC1 complex that increases, through p70S6K activation, local protein synthesis that is necessary for IA LTM memory (Lana et al., 2013). A crosstalk between ERK and mTOR at different levels of the signalling flow is shown. Indeed, the mitogen-activated protein kinases have also been shown to regulate mTORC1. ERK was found to phosphorylate and inhibit the function of TSC2, albeit through different mechanisms and at different phosphorylation sites (Corradetti & Guan, 2006). For instance, a recent study (Tsokas, Ma, Iyengar, Landau, & Blitzer, 2007) showed an interesting interplay between ERK and mTOR pathways at CA3–CA1 synapses: ERK is required for the high frequency stimulation-induced activation of the mTOR pathway in the hippocampus. Further studies demonstrate a complex interplay among the cholinergic system, ERK and mTOR. For instance, mTOR is known to phosphorylate p70S6K at the site Thr389, while ERK is able to phosphorylate p70S6K at the site Thr421/Ser424 (Lafay-Chebassier et al., 2006). It was shown that the mAChRs agonist oxotremorine given IP induces phosphorylation of p70S6K at Thr389, which is not dependent upon activation of mTOR but possibly upon the ERK pathway activation (Deguil et al., 2008). Indeed, in a previous study, it was shown that mTOR could phosphorylate p70S6K at Thr421/Ser424, a specific site of ERK and inversely, ERK could phosphorylate p70S6K at Thr389 controlled by mTOR signalling (Lafay-Chebassier et al., 2006), making the story even more complex. It is also currently accepted that ERK and mTORC1 synergistically regulate Eukaryotic translation initiation factor 4E (eIF4E) and translation initiation in LTM and synaptic plasticity (Panja et al., 2009; Gal-Ben-Ari & Rosenblum, 2012).

It seems therefore that independent/concurrent/synergistic recruitment and activation of ERK and mTOR signalling cascades may be a conserved mechanism for the precise regulation of translation downstream of various neuromodulatory receptors.

5. Conclusions

The fascinating question on how and where memories are formed in our brain is the focus of intense investigations, and although a few answers are now available, we are still far from having a complete understanding of the process. In this review we summarized the present knowledge on the complex involvement of ACh, ERK and mTOR in the hippocampal mechanisms of IA memory. However, it must be kept in mind that other neurotransmitter systems and other signalling pathways are involved in the formation of IA memories. It should also be pointed out that the hippocampal structure is more complex than originally thought. Indeed, it has been demonstrated that the dorsal hippocampus has different functions from the ventral hippocampus in memory formation (Kheirbek et al., 2013). Furthermore, it is still a matter of investigation whether STM and LTM proceed in series, or in parallel. It has been described in rodent models that *de novo* protein synthesis is required to stabilize a STM into a LTM (Abel & Lattal, 2001), whereas others (Marti, Ramirez, Dos Reis, & Izquierdo, 2004) claim that STM and LTM are not processed by separate mechanisms. Nonetheless, the present view is that different molecular mechanisms may be needed to form STM and LTM (Lana et al., 2013), whereas some mechanisms are involved in both (Izquierdo et al., 1998a). Indeed, the demonstration that STM and LTM, acquired in a one-trial IA task, are independent phenomena, is given by the findings that pharmacological treatments block STM independently from LTM (Izquierdo et al., 1998a; Izquierdo, Medina, Vianna, Izquierdo, & Barros, 1999; Vianna, Izquierdo, Barros, Medina, & Izquierdo, 1999; Vianna et al., 2000; Izquierdo et al., 2002; Lana et al., 2013). Finally, with Tranel and Damasio (1995) we could conclude that “we have barely begun to unravel some of the mysteries of how our brains sub serve memory”.

5. Legend to Figures

Figure 1. A schematic overview, based upon the literature and our own published work, of the intracellular pathways activated by mAChR and nAChR leading to STM and LTM IA encoding. In this scheme are also indicated the sites of action of the principal pharmacological tools used in the researches reviewed in this paper. Direct activation is represented by continuous arrows, indirect activation by dotted arrows. Black arrows indicate activation, T-shaped arrows indicate inhibition; open arrow indicates ACh release. IA: inhibitory avoidance STM: short term memory; LTM: long term memory.

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7. References

- Abel, T., & Lattal, K. M. (2001). Molecular mechanisms of memory acquisition, consolidation and retrieval. *Current Opinion in Neurobiology*, *11*(2), 180-187.
- Aberg, M. A., Aberg, N. D., Palmer, T. D., Alborn, A. M., Carlsson-Skwirut, C., Bang, P. et al. (2003). IGF-I has a direct proliferative effect in adult hippocampal progenitor cells. *Molecular and Cellular Neuroscience*, *24*, 23-40.
- Adams, J. P., & Sweatt, J. D. (2002). Molecular psychology: roles for the ERK MAP kinase cascade in memory. *Annual Review of Pharmacology and Toxicology*, *42*, 135-163.
- Alkondon, M., & Albuquerque, E. X. (1993). Diversity of Nicotinic Acetylcholine-Receptors in Rat Hippocampal-Neurons .1. Pharmacological and Functional Evidence for Distinct Structural Subtypes. *Journal of Pharmacology and Experimental Therapeutics*, *265*, 1455-1473.
- Alkondon, M., & Albuquerque, E. X. (2001). Nicotinic acetylcholine receptor alpha7 and alpha4 beta2 subtypes differentially control GABAergic input to CA1 neurons in rat hippocampus. *Journal of Neurophysiology*, *86*, 3043-3055.
- Alkondon, M., & Albuquerque, E. X. (2004). The nicotinic acetylcholine receptor subtypes and their function in the hippocampus and cerebral cortex. *Acetylcholine in the Cerebral Cortex*, *145*, 109-120.
- Alonso, M., Viola, H., Izquierdo, I., & Medina, J. H. (2002). Aversive experiences are associated with a rapid and transient activation of ERKs in the rat hippocampus. *Neurobiology of Learning and Memory*, *77*, 119-124.
- Apergis-Schoute, A. M., Debiec, J., Doyere, V., LeDoux, J. E., & Schafe, G. E. (2005). Auditory fear conditioning and long-term potentiation in the lateral amygdala require ERK/MAP kinase signaling in the auditory thalamus: a role for presynaptic plasticity in the fear system. *Journal of Neuroscience*, *25*, 5730-5739.
- Arvais, E. W., Romanelli, A., Hou, X., & Davis, J. S. (2006). AKT-independent phosphorylation of TSC2 and activation of mTOR and ribosomal protein S6 kinase signaling by prostaglandin F2alpha. *Journal of Biological Chemistry*, *281*, 26904-26913.
- Atkins, C. M., Selcher, J. C., Petraitis, J. J., Trzaskos, J. M., & Sweatt, J. D. (1998). The MAPK cascade is required for mammalian associative learning. *Nature Neuroscience*, *1*, 602-609.
- Bading, H., & Greenberg, M. E. (1991). Stimulation of protein tyrosine phosphorylation by NMDA receptor activation. *Science*, *253*, 912-914.

- Bailey, C. H., Kandel, E. R., & Si, K. (2004). The persistence of long-term memory: a molecular approach to self-sustaining changes in learning-induced synaptic growth. *Neuron*, *44*, 49-57.
- Baratti, C. M., Huygens, P., Mino, J., Merlo, A., & Gardella, J. (1979). Memory facilitation with posttrial injection of oxotremorine and physostigmine in mice. *Psychopharmacology (Berl)*, *64*, 85-88.
- Barros, D. M., Pereira, P., Medina, J. H., & Izquierdo, I. (2002). Modulation of working memory and of long- but not short-term memory by cholinergic mechanisms in the basolateral amygdala. *Behavioural Pharmacology*, *13*, 163-167.
- Bartolini, L., Risaliti, R., & Pepeu, G. (1992). Effect of scopolamine and nootropic drugs on rewarded alternation in a T-maze. *Pharmacology Biochemistry and Behavior*, *43*, 1161-1164.
- Baxter, M. G., Bucci, D. J., Sobel, T. J., Williams, M. J., Gorman, L. K., & Gallagher, M. (1996) Intact spatial learning following lesions of basal forebrain cholinergic neurons. *Neuroreport*, *7*, 1417-1420.
- Bekinschtein, P., Katche, C., Slipczuk, L., Gonzalez, C., Dorman, G., Cammarota, M. et al. (2010). Persistence of long-term memory storage: new insights into its molecular signatures in the hippocampus and related structures. *Neurotoxicity Research*, *18*, 377-385.
- Bekinschtein, P., Katche, C., Slipczuk, L. N., Igaz, L. M., Cammarota, M., Izquierdo, I. et al. (2007). mTOR signaling in the hippocampus is necessary for memory formation. *Neurobiology of Learning and Memory*, *87*, 303-307.
- Belelovsky, K., Kaphzan, H., Elkobi, A., & Rosenblum, K. (2009). Biphasic activation of the mTOR pathway in the gustatory cortex is correlated with and necessary for taste learning. *Journal of Neuroscience*, *29*, 7424-7431.
- Bell, K. A., Shim, H., Chen, C. K., & McQuiston, A. R. (2011). Nicotinic excitatory postsynaptic potentials in hippocampal CA1 interneurons are predominantly mediated by nicotinic receptors that contain alpha4 and beta2 subunits. *Neuropharmacology*, *61*, 1379-1388.
- Bellucci, A., Luccarini, I., Scali, C., Prosperi, C., Giovannini, M. G., Pepeu, G. et al. (2006). Cholinergic dysfunction, neuronal damage and axonal loss in TgCRND8 mice. *Neurobiology of Disease*, *23*, 260-272.
- Berg, D. K., & Conroy, W. G. (2002). Nicotinic alpha 7 receptors: synaptic options and downstream signaling in neurons. *Journal of Neurobiology*, *53*, 512-523.
- Berger, S. L., Kouzarides, T., Shiekhatar, R., & Shilatifard, A. (2009) An operational definition of epigenetics. *Genes & Development*, *23*, 781-783.
- Berkeley, J. L., Gomeza, J., Wess, J., Hamilton, S. E., Nathanson, N. M., & Levey, A. I. (2001). M1 muscarinic acetylcholine receptors activate extracellular signal-regulated kinase in CA1 pyramidal neurons in mouse hippocampal slices. *Molecular and Cellular Neuroscience*, *18*, 512-524.

- Berman, D. E., Hazvi, S., Rosenblum, K., Seger, R., & Dudai, Y. (1998). Specific and differential activation of mitogen-activated protein kinase cascades by unfamiliar taste in the insular cortex of the behaving rat. *Journal of Neuroscience*, *18*, 10037-10044.
- Bevilaqua, L. R., Bonini, J. S., Rossato, J. I., Izquierdo, L. A., Cammarota, M., & Izquierdo, I. (2006). The entorhinal cortex plays a role in extinction. *Neurobiology of Learning and Memory*, *85*, 192-197.
- Bhalla, U. S., & Iyengar, R. (1999). Emergent properties of networks of biological signaling pathways. *Science*, *283*, 381-387.
- Bianchi, L., Ballini, C., Colivicchi, M. A., Della, C. L., Giovannini, M. G., & Pepeu, G. (2003). Investigation on acetylcholine, aspartate, glutamate and GABA extracellular levels from ventral hippocampus during repeated exploratory activity in the rat. *Neurochemical Research*, *28*, 565-573.
- Blokland, A., Honig, W., & Raaijmakers, W. G. M. (1992). Effects of Intrahippocampal Scopolamine Injections in A Repeated Spatial Acquisition Task in the Rat. *Psychopharmacology*, *109*, 373-376.
- Blum, S., Moore, A. N., Adams, F., & Dash, P. K. (1999). A mitogen-activated protein kinase cascade in the CA1/CA2 subfield of the dorsal hippocampus is essential for long-term spatial memory. *Journal of Neuroscience*, *19*, 3535-3544.
- Boccia, M. M., Acosta, G. B., Blake, M. G., & Baratti, C. M. (2004). Memory consolidation and reconsolidation of an inhibitory avoidance response in mice: Effects of ICV injections of hemicholinium-3. *Neuroscience*, *124*, 735-741.
- Bolduc, F. V., Bell, K., Cox, H., Broadie, K. S., & Tully, T. (2008). Excess protein synthesis in *Drosophila* Fragile X mutants impairs long-term memory. *Nature Neuroscience*, *11*, 1143-1145.
- Boschert, U., Dickinson, R., Muda, M., Camps, M., & Arkininstall, S. (1998). Regulated expression of dual specificity protein phosphatases in rat brain. *NeuroReport*, *9*, 4081-4086.
- Boulton, T. G., Yancopoulos, G. D., Gregory, J. S., Slaughter, C., Moomaw, C., Hsu, J. et al. (1990). An insulin-stimulated protein kinase similar to yeast kinases involved in cell cycle control. *Science*, *249*, 64-67.
- Bourtchouladze, R., Abel, T., Berman, N., Gordon, R., Lapidus, K., & 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. *Learning & Memory*, *5*, 365-374.
- Bove, J., Martinez-Vicente, M., & Vila, M. (2011). Fighting neurodegeneration with rapamycin: mechanistic insights. *Nature Reviews Neuroscience*, *12*, 437-452.
- Brown, D. A., & Adams, P. R. (1980). Muscarinic suppression of a novel voltage-sensitive K⁺ current in a vertebrate neurone. *Nature*, *283*, 673-676.
- Brown, D. A. (2010). Muscarinic Acetylcholine Receptors (mAChRs) in the Nervous System: Some Functions and Mechanisms. *Journal of Molecular Neuroscience*, *41*, 340-346.

- Buchanan, K. A., Petrovic, M. M., Chamberlain, S. E., Marrion, N. V., & Mellor, J. R. (2010). Facilitation of long-term potentiation by muscarinic M(1) receptors is mediated by inhibition of SK channels. *Neuron*, *68*, 948–963.
- Cammalleri, M., Lutjens, R., Berton, F., King, A. R., Simpson, C., Francesconi, W. et al. (2003). Time-restricted role for dendritic activation of the mTOR-p70(S6K) pathway in the induction of late-phase long-term potentiation in the CA1. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 14368-14373.
- Cammarota, M., Bevilaqua, L. R., Ardenghi, P., Paratcha, G., Levi, d. S., 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. *Molecular Brain Research*, *76*, 36-46.
- Cammarota, M., Bevilaqua, L. R. M., Medina, J. H., & Izquierdo, I. 2007. Studies of Short-Term Avoidance Memory. In Bermudez-Rattoni, F. (Ed.) *Frontiers in Neuroscience. Neural Plasticity and Memory: From Genes to Brain Imaging*. Boca Raton (FL): CRC Press.
- Casadio, A., Martin, K. C., Giustetto, M., Zhu, H. X., Chen, M., Bartsch, D. et al. (1999). A transient, neuron-wide form of CREB-mediated long-term facilitation can be stabilized at specific synapses by local protein synthesis. *Cell*, *99*, 221-237.
- Castillo, D. V., & Escobar, M. L. (2011). A role for MAPK and PI-3K signaling pathways in brain-derived neurotrophic factor modification of conditioned taste aversion retention. *Behavioural Brain Research*, *217*, 248-252.
- Cestari, V., Costanzi, M., Castellano, C., & Rossi-Arnaud, C. (2006). A role for ERK2 in reconsolidation of fear memories in mice. *Neurobiology of Learning and Memory*, *86*, 133-143.
- Chan, G. P., Wu, E. H., & Wong, Y. H. (2009). Regulation of mTOR and p70 S6 kinase by the muscarinic M4 receptor in PC12 cells. *Cell Biology International*, *33*, 230-238.
- Chang, L., & Karin, M. (2001). Mammalian MAP kinase signalling cascades. *Nature*, *410*, 37-40.
- Chen, H. J., Rojas-Soto, M., Oguni, A., & Kennedy, M. B. (1998). A synaptic Ras-GTPase activating protein (p135 SynGAP) inhibited by CaM kinase II. *Neuron*, *20*, 895-904.
- Chishti, M. A., Yang, D. S., Janus, C., Phinney, A. L., Horne, P., Pearson, J. et al. (2001). Early-onset amyloid deposition and cognitive deficits in transgenic mice expressing a double mutant form of amyloid precursor protein 695. *Journal of Biological Chemistry*, *276*, 21562-21570.
- Choo, A. Y., Yoon, S. O., Kim, S. G., Roux, P. P., & Blenis, J. (2008). Rapamycin differentially inhibits S6Ks and 4E-BP1 to mediate cell-type-specific repression of mRNA translation. *Proceedings of the National Academy of Sciences of the United States of America*, *105*, 17414-17419.

- Chwang, W. B., Arthur, J. S., Schumacher, A., & Sweatt, J. D. (2007). The nuclear kinase mitogen- and stress-activated protein kinase 1 regulates hippocampal chromatin remodeling in memory formation. *Journal of Neuroscience*, *27*, 12732-12742.
- Chwang, W. B., O'Riordan, K. J., Levenson, J. M., & Sweatt, J. D. (2006). ERK/MAPK regulates hippocampal histone phosphorylation following contextual fear conditioning. *Learning & Memory*, *13*, 322-328.
- Cole, A. E., & Nicoll, R. A. (1984). Characterization of a slow cholinergic post-synaptic potential recorded *in vitro* from rat hippocampal pyramidal cells. *The Journal of Physiology (London)*, *352*, 173-188.
- Corbit, K. C., Foster, D. A., & Rosner, M. R. (1999). Protein kinase Cdelta mediates neurogenic but not mitogenic activation of mitogen-activated protein kinase in neuronal cells. *Molecular and Cellular Biology*, *19*, 4209-4218.
- Corradetti, M. N., & Guan, K. L. (2006). Upstream of the mammalian target of rapamycin: do all roads pass through mTOR? *Oncogene*, *25*, 6347-6360.
- Cruz-Morales, S. E., Duran-Arevalo, M., Diaz Del Guante, M. A., Quirarte, G., & Prado-Alcalá, R.A. (1992). A threshold for the protective effect of over-reinforced passive avoidance against scopolamine-induced amnesia. *Behavioral and Neural Biology*, *57*, 256-259.
- Cutuli, D., De Bartolo, P., Caporali, P., Tartaglione, A.M, Oddi, D., D'Amato, et al., (2013) Neuroprotective effects of donepezil against cholinergic depletion. *Alzheimer's Research Therapy*, *5*, 50-68.
- Dash, P. K., Orsi, S. A., & Moore, A. N. (2006). Spatial memory formation and memory-enhancing effect of glucose involves activation of the tuberous sclerosis complex-Mammalian target of rapamycin pathway. *Journal of Neuroscience*, *26*, 8048-8056.
- Dasari, S., & Gullledge, A. T.(2011). M1 and M4 receptors modulate hippocampal pyramidal neurons. *Journal of Neurophysiology*, *105*, 779-792.
- Davis, H. P., & Squire, L. R. (1984). Protein synthesis and memory: a review. *Psychology Bulletin*, *96*, 518-559.
- Davis, S., Vanhoutte, P., Pages, C., Caboche, J., & Laroche, S. (2000). The MAPK/ERK cascade targets both Elk-1 and cAMP response element-binding protein to control long-term potentiation-dependent gene expression in the dentate gyrus *in vivo*. *Journal of Neuroscience*, *20*, 4563-4572.
- de Bruin, N. M., Ellenbroek, B. A., van Luijckelaar, E. L., Cools, A. R., & Stevens K. E. (2001) Hippocampal and cortical sensory gating in rats: Effects of quinpirole microinjections in nucleus accumbens core and shell. *Neuroscience*, *105*:169-180
- Decker, M. W., Brioni, J. D., Bannon, A. W., & Arneric, S. P. (1995). Diversity of neuronal nicotinic acetylcholine receptors: lessons from behavior and implications for CNS therapeutics. *Life Sciences*, *56*, 545-570.

- Deguil, J., Perault-Pochat, M. C., Chavant, F., Lafay-Chebassier, C., Fauconneau, B., & Pain, S. (2008). Activation of the protein p70S6K via ERK phosphorylation by cholinergic muscarinic receptors stimulation in human neuroblastoma cells and in mice brain. *Toxicology Letters*, *182*, 91-96.
- Demeter, E., & Sarter, M. (2013). Leveraging the cortical cholinergic system to enhance attention. *Neuropharmacology*, *64*, 294-304.
- Dineley, K. T., Westerman, M., Bui, D., Bell, K., Ashe, K. H., & Sweatt, J. D. (2001). Beta-amyloid activates the mitogen-activated protein kinase cascade via hippocampal alpha7 nicotinic acetylcholine receptors: In vitro and in vivo mechanisms related to Alzheimer's disease. *Journal of Neuroscience*, *21*, 4125-4133.
- Douglas, C. L., Baghdoyan, H. A., & Lydic, R. (2002). Prefrontal cortex acetylcholine release, EEG slow waves, and spindles are modulated by M2 autoreceptors in C57BL/6J mouse. *Journal of Neurophysiology*, *87*, 2817-2822.
- Drutel, G., Peitsaro, N., Karlstedt, K., Wieland, K., Smit, M. J., Timmerman, H. et al. (2001). Identification of rat H3 receptor isoforms with different brain expression and signaling properties. *Molecular Pharmacology*, *59*, 1-8.
- Easton, A., Fitchett, A. E., Eacott, M. J., & Baxter, M. G. (2011). Medial Septal Cholinergic Neurons Are Necessary for Context-Place Memory But Not Episodic-like Memory. *Hippocampus*, *21*, 1021-1027
- Ehninger, D., Han, S., Shilyansky, C., Zhou, Y., Li, W. D., Kwiatkowski, D. J. et al. (2008). Reversal of learning deficits in a Tsc2(+/-) mouse model of tuberous sclerosis. *Nature Medicine*, *14*, 843-848.
- Eichenbaum, H. (2001). The hippocampus and declarative memory: cognitive mechanisms and neural codes. *Behavioral Brain Research*, *127*, 199-207.
- Eidi, M., Zarrindast, M. R., Eidi, A., Oryan, S., & Parivar, K. (2003). Effects of histamine and cholinergic systems on memory retention of passive avoidance learning in rats. *European Journal of Pharmacology*, *465*, 91-96.
- English, J. D., & Sweatt, J. D. (1996). Activation of p42 mitogen-activated protein kinase in hippocampal long term potentiation. *Journal of Biological Chemistry*, *271*, 24329-24332.
- English, J. D., & Sweatt, J. D. (1997). A requirement for the mitogen-activated protein kinase cascade in hippocampal long term potentiation. *Journal of Biological Chemistry*, *272*, 19103-19106.
- Enslin, H., & Davis, R. J. (2001). Regulation of MAP kinases by docking domains. *Biology of the Cell*, *93*, 5-14.
- Estape, N., & Steckler, T. (2002). Cholinergic blockade impairs performance in operant DNMT in two inbred strains of mice. *Pharmacology Biochemistry and Behavior*, *72*, 319-334.
- Everitt, B. J., & Robbins, T. W. (1997). Central cholinergic system and cognition. *Annual Review of Psychology*, *48*, 649-684.

- Feig, S., & Lipton, P. (1993). Pairing the cholinergic agonist carbachol with patterned Schaffer collateral stimulation initiates protein synthesis in hippocampal CA1 pyramidal cell dendrites via a muscarinic, NMDA-dependent mechanism. *Journal of Neuroscience*, *13*, 1010-1021.
- Felix, R., & Levin, E. D. (1997). Nicotinic antagonist administration into the ventral hippocampus and spatial working memory in rats. *Neuroscience*, *81*, 1009-1017.
- Fernandez De Sevilla, D., Nunez, A., Borde, M., Malinow, R., & Buno, W. (2008). Cholinergic-mediated IP3-receptor activation induces long-lasting synaptic enhancement in CA1 pyramidal neurons. *Journal of Neuroscience*, *28*, 1469-1478.
- Fiore, R. S., Bayer, V. E., Pelech, S. L., Posada, J., Cooper, J. A., & Baraban, J. M. (1993). p42 mitogen-activated protein kinase in brain: prominent localization in neuronal cell bodies and dendrites. *Neuroscience*, *55*, 463-472.
- Fiore, R. S., Murphy, T. H., Sanghera, J. S., Pelech, S. L., & Baraban, J. M. (1993). Activation of p42 mitogen-activated protein kinase by glutamate receptor stimulation in rat primary cortical cultures. *Journal of Neurochemistry*, *61*, 1626-1633.
- Flood, D. G., Finn, J. P., Walton, K. M., Dionne, C. A., Contreras, P. C., Miller, M. S. et al. (1998). Immunolocalization of the mitogen-activated protein kinases p42MAPK and JNK1, and their regulatory kinases MEK1 and MEK4, in adult rat central nervous system. *Journal of Comparative Neurology*, *398*, 373-392.
- Frazier, C. J., Rollins, Y. D., Breese, C. R., Leonard, S., Freedman, R., & Dunwiddie, T. V. (1998). Acetylcholine activates an alpha-bungarotoxin-sensitive nicotinic current in rat hippocampal interneurons, but not pyramidal cells. *Journal of Neuroscience*, *18*, 1187-1195.
- Freeman, F. M., Rose, S. P., & Scholey, A. B. (1995). Two time windows of anisomycin-induced amnesia for passive avoidance training in the day-old chick. *Neurobiology of Learning and Memory*, *63*, 291-295.
- Frotscher, M., & Leranth, C. (1985). Cholinergic Innervation of the Rat Hippocampus As Revealed by Choline-Acetyltransferase Immunocytochemistry - A Combined Light and Electron-Microscopic Study. *Journal of Comparative Neurology*, *239*, 237-246.
- Fu, W. M., Liou, H. C., & Chen, Y. H. (1998). Nerve terminal currents induced by autoreception of acetylcholine release. *Journal of Neuroscience*, *18*, 9954-9961.
- Gal-Ben-Ari, S., & Rosenblum, K. (2012). Molecular mechanisms underlying memory consolidation of taste information in the cortex. *Frontiers in Behavioral Neuroscience*, *5*, 87-101.
- Garcia-Alloza, M., Zaldua, N., Diez-Ariza, M., Marcos, B., Lasheras, B., Gil-Bea, F. J. et al. (2006). Effect of Selective Cholinergic Denervation on the Serotonergic System: Implications for Learning and Memory. *Journal of Neuropathology & Experimental Neurology*, *65*, 1074-1081.
- Gardiner, J. E. (1961). The inhibition of acetylcholine synthesis in brain by a hemicholinium. *Biochemical Journal*, *81*, 297-303.

- Geetha N., Mihaly J., Stockenhuber, A., Blasi, F., Uhrin, P., Binder, B. R., et al. (2011). Signal integration and coincidence detection in the mitogen-activated protein kinase/extracellular signal-regulated kinase (ERK) cascade: concomitant activation of receptor tyrosine kinases and of LRP-1 leads to sustained ERK phosphorylation via down-regulation of dual specificity phosphatases (DUSP1 and -6). *The Journal of Biological Chemistry*, *286*, 25663-25674.
- Giessel, A. J., & Sabatini, B. L. (2010). M1 muscarinic receptors boost synaptic potentials and calcium influx in dendritic spines by inhibiting postsynaptic SK channels. *Neuron*, *68*, 936-947.
- Giovannini, M. G., Bartolini, L., Bacciottini, L., Greco, L., & Blandina, P. (1999). Effects of histamine H3 receptor agonists and antagonists on cognitive performance and scopolamine-induced amnesia. *Behavioral Brain Research*, *104*, 147-155.
- Giovannini, M. G., Blitzler, R. D., Wong, T., Asoma, K., Tsokas, P., Morrison, J. H. et al. (2001a). Mitogen-activated protein kinase regulates early phosphorylation and delayed expression of Ca²⁺/calmodulin-dependent protein kinase II in long-term potentiation. *Journal of Neuroscience*, *21*, 7053-7062.
- Giovannini, M. G., Cerbai, F., Bellucci, A., Melani, C., Grossi, C., Bartolozzi, C. et al. (2008). Differential activation of mitogen-activated protein kinase signalling pathways in the hippocampus of CRND8 transgenic mouse, a model of Alzheimer's disease. *Neuroscience*, *153*, 618-633.
- Giovannini, M. G., Efoudebe, M., Passani, M. B., Baldi, E., Bucherelli, C., Giachi, F. et al. (2003). Improvement in fear memory by histamine-elicited ERK2 activation in hippocampal CA3 cells. *Journal of Neuroscience*, *23*, 9016-9023.
- Giovannini, M. G., Pazzagli, M., Malmberg-Aiello, P., Della, C. L., Rakovska, A. D., Cerbai, F. et al. (2005). Inhibition of acetylcholine-induced activation of extracellular regulated protein kinase prevents the encoding of an inhibitory avoidance response in the rat. *Neuroscience*, *136*, 15-32.
- Giovannini, M. G., Rakovska, A., Benton, R. S., Pazzagli, M., Bianchi, L., & Pepeu, G. (2001b). Effects of novelty and habituation on acetylcholine, GABA, and glutamate release from the frontal cortex and hippocampus of freely moving rats. *Neuroscience*, *106*, 43-53.
- Givens, B., & Olton, D. S. (1995). Bidirectional modulation of scopolamine-induced working memory impairments by muscarinic activation of the medial septal area. *Neurobiology of Learning and Memory*, *63*, 269-276.
- Gold, P. E. (1986). The Use of Avoidance Training in Studies of Modulation of Memory Storage. *Behavioral and Neural Biology*, *46*, 87-98.
- Gonzales, K. K., Pare, J. F., Wichmann, T., & Smith, Y. (2013). GABAergic inputs from direct and indirect striatal projection neurons onto cholinergic interneurons in the primate putamen. *Journal of Comparative Neurology*, *521*, 2502-2522.
- Gould, T. J., & Leach, P. (2014). Cellular, molecular, and genetic substrates underlying the impact of nicotine on learning. *Neurobiology of Learning and Memory*, *107*, 108-132.

- Gould, T. J., Wilkinson, D. S., Yildirim, E., Poole, R. L., Leach, P. T., & Simmons, S. J. (2014). Nicotine shifts the temporal activation of hippocampal protein kinase A and extracellular signal-regulated kinase 1/2 to enhance long-term, but not short-term, hippocampus-dependent memory. *Neurobiology of Learning and Memory*, *109*, 151-159.
- Graber, T. E., McCamphill, P. K., & Sossin, W. S. (2013). A recollection of mTOR signaling in learning and memory. *Learning & Memory*, *20*, 518-530.
- Graef, S., Schoknecht, P., Sabri, O., & Hegerl, U. (2011). Cholinergic receptor subtypes and their role in cognition, emotion, and vigilance control: An overview of preclinical and clinical findings. *Psychopharmacology*, *215*, 205-229.
- Graff, J., Kim, D., Dobbin, M. M., & Tsai, L. H. (2011). Epigenetic regulation of gene expression in physiological and pathological brain processes. *Physiological Reviews*, *91*, 603-649.
- Grewal, S. S., York, R. D., & Stork, P. J. (1999). Extracellular-signal-regulated kinase signalling in neurons. *Current Opinion in Neurobiology*, *9*, 544-553.
- Gu, Z., & Yakel, J. L. (2011). Timing-dependent septal cholinergic induction of dynamic hippocampal synaptic plasticity. *Neuron*, *71*, 155-165.
- Gulledge, A. T., & Kawaguchi, Y. (2007). Phasic cholinergic signaling in the hippocampus: functional homology with the neocortex. *Hippocampus*, *17*, 327-332.
- Gutkind, J. S. (1998). The pathways connecting G protein-coupled receptors to the nucleus through divergent mitogen-activated protein kinase cascades. *Journal of Biological Chemistry*, *273*, 1839-1842.
- Halliwel, J. V., & Adams, P. R. (1982). Voltage-clamp analysis of muscarinic excitation in hippocampal neurons. *Brain Research*, *250*, 71-92.
- Harburger, L.L., Saadi, A., & Frick, K.M. (2009). Dose-dependent effects of post-training estradiol plus progesterone treatment on object memory consolidation and hippocampal extracellular signal-regulated kinase activation in young ovariectomized mice. *Behavioural Neuroscience*, *160*, 6-12.
- Hasselmo, M. E., Wyble, B. P., & Wallenstein, G. V. (1996). Encoding and retrieval of episodic memories: role of cholinergic and GABAergic modulation in the hippocampus. *Hippocampus*, *6*(6), 693-708.
- Hay, N., & Sonenberg, N. (2004). Upstream and downstream of mTOR. *Genes & Development*, *18*, 1926-1945.
- Hetman, M., Kanning, K., Cavanaugh, J. E., & Xia, Z. (1999). Neuroprotection by brain-derived neurotrophic factor is mediated by extracellular signal-regulated kinase and phosphatidylinositol 3-kinase. *Journal of Biological Chemistry*, *274*, 22569-22580.
- Hoeffler, C. A., & Klann, E. (2010). mTOR signaling: at the crossroads of plasticity, memory and disease. *Trends in Neurosciences*, *33*, 67-75.

- Hoeffler, C. A., Tang, W., Wong, H., Santillan, A., Patterson, R. J., Martinez, L. A. et al. (2008). Removal of FKBP12 enhances mTOR-Raptor interactions, LTP, memory, and perseverative/repetitive behavior. *Neuron*, *60*, 832-845.
- Husi, H., Ward, M. A., Choudhary, J. S., Blackstock, W. P., & Grant, S. G. (2000). Proteomic analysis of NMDA receptor-adhesion protein signaling complexes. *Nature Neuroscience*, *3*, 661-669.
- Igaz, L. M., Bekinschtein, P., Izquierdo, I., & Medina, J. H. (2004). One-trial aversive learning induces late changes in hippocampal CaMKIIalpha, Homer 1a, Syntaxin 1a and ERK2 protein levels. *Brain Research Molecular Brain Research*, *132*, 1-12.
- Igaz, L. M., Vianna, M. R. M., Medina, J. H., & Izquierdo, I. (2002). Two time periods of hippocampal mRNA synthesis are required for memory consolidation of fear-motivated learning. *Journal of Neuroscience*, *22*, 6781-6789.
- Impey, S., Obrietan, K., Wong, S. T., Poser, S., Yano, S., Wayman, G. et al. (1998). Cross talk between ERK and PKA is required for Ca²⁺ stimulation of CREB-dependent transcription and ERK nuclear translocation. *Neuron*, *21*, 869-883.
- Introini Collison, I. B., & Baratti, C. M. (1992). Memory-Modulatory Effects of Centrally Acting Noradrenergic Drugs - Possible Involvement of Brain Cholinergic Mechanisms. *Behavioral and Neural Biology*, *57*, 248-255.
- Izquierdo, I. (1989). Mechanism of action of scopolamine as an amnesic. *Trends in Pharmacological Sciences*, *10*, 175-177.
- Izquierdo, I., da Cunha, C., Rosat, R., Jerusalinsky, D., Ferreira, M. B., & Medina, J. H. (1992). Neurotransmitter receptors involved in post-training memory processing by the amygdala, medial septum, and hippocampus of the rat. *Behavioral and Neural Biology*, *58*, 16-26.
- Izquierdo, I., Barros, D. M., Mello e Souza, de Souza, M. M., Izquierdo, L. A., & Medina, J. H. (1998a). Mechanisms for memory types differ. *Nature*, *393*, 635-636.
- Izquierdo, I., Izquierdo, L. A., Barros, D. M., Mello e Souza, de Souza, M. M., Quevedo, J. et al. (1998b). Differential involvement of cortical receptor mechanisms in working, short-term and long-term memory. *Behavioural Pharmacology*, *9*, 421-427.
- Izquierdo, I., & Medina, J. H. (1997). Memory formation: The sequence of biochemical events in the hippocampus and its connection to activity in other brain structures. *Neurobiology of Learning and Memory*, *68*, 285-316.
- Izquierdo, I., Medina, J. H., Izquierdo, L. A., Barros, D. M., de Souza, M. M., & Mello e Souza (1998c). Short- and long-term memory are differentially regulated by monoaminergic systems in the rat brain. *Neurobiology of Learning and Memory*, *69*, 219-224.
- Izquierdo, I., Medina, J. H., Vianna, M. R., Izquierdo, L. A., & Barros, D. M. (1999). Separate mechanisms for short- and long-term memory. *Behavioral Brain Research*, *103*, 1-11.

- Izquierdo, I., Quillfeldt, J. A., Zanatta, M. S., Quevedo, J., Schaeffer, E., Schmitz, P. K. et al. (1997). Sequential role of hippocampus and amygdala, entorhinal cortex and parietal cortex in formation and retrieval of memory for inhibitory avoidance in rats. *European Journal of Neuroscience*, *9*, 786-793.
- Izquierdo, L. A., Barros, D. M., Ardenghi, P. G., Pereira, P., Rodrigues, C., Choi, H. et al. (2000). Different hippocampal molecular requirements for short- and long-term retrieval of one-trial avoidance learning. *Behavioral Brain Research*, *111*, 93-98.
- Izquierdo, L. A., Barros, D. M., da Costa, J. C., Furini, C., Zinn, C., Carnmarota, M. et al. (2007). A link between role of two prefrontal areas in immediate memory and in long-term memory consolidation. *Neurobiology of Learning and Memory*, *88*, 160-166.
- Izquierdo, L. A., Barros, D. M., Vianna, M. R., Coitinho, A., deDavid e Silva, Choi, H. et al. (2002). Molecular pharmacological dissection of short- and long-term memory. *Cellular and Molecular Neurobiology*, *22*, 269-287.
- Janis, L. S., Glasier, M. M., Fulop, Z., & Stein, D. G. (1998). Intraseptal injections of 192 IgG saporin produce deficits for strategy selection in spatial-memory tasks. *Behavioural Brain Research*, *90*, 23-34.
- Jeruzalinsky, D., Cervenansky, C., Walz, R., Bianchin, M., & Izquierdo, I. (1993). A peptide muscarinic toxin from the Green Mamba venom shows agonist-like action in an inhibitory avoidance learning task. *European Journal of Pharmacology*, *240*, 103-105.
- Ji, D. Y., & Dani, J. A. (2000). Inhibition and disinhibition of pyramidal neurons by activation of nicotinic receptors on hippocampal interneurons. *Journal of Neurophysiology*, *83*, 2682-2690.
- Kaminska, B., Kaczmarek, L., Zangenehpour, S., & Chaudhuri, A. (1999). Rapid phosphorylation of Elk-1 transcription factor and activation of MAP kinase signal transduction pathways in response to visual stimulation. *Molecular and Cellular Neuroscience*, *13*, 405-414.
- Kanterewicz, B. I., Urban, N. N., McMahon, D. B., Norman, E. D., Giffen, L. J., Favata, M. F. et al. (2000). The extracellular signal-regulated kinase cascade is required for NMDA receptor-independent LTP in area CA1 but not area CA3 of the hippocampus. *Journal of Neuroscience*, *20*, 3057-3066.
- Kawai, H., Zago, W., & Berg, D. K. (2002). Nicotinic alpha7 receptor clusters on hippocampal GABAergic neurons: regulation by synaptic activity and neurotrophins. *Journal of Neuroscience*, *22*, 7903-7912.
- Kelleher, R. J., III, Govindarajan, A., Jung, H. Y., Kang, H., & Tonegawa, S. (2004). Translational control by MAPK signaling in long-term synaptic plasticity and memory. *Cell*, *116*, 467-479.
- Kenney, J. W., Florian, C., Portugal, G. S., Abel, T., & Gould, T. J. (2010). Involvement of Hippocampal Jun-N Terminal Kinase Pathway in the Enhancement of Learning and Memory by Nicotine. *Neuropsychopharmacology*, *35*, 483-492.

- Kenney, J. W., Poole, R. L., Adoff, M. D., Logue, S. F., & Gould, T. J. (2012). Learning and Nicotine Interact to Increase CREB Phosphorylation at the jnk1 Promoter in the Hippocampus. *Plos One*, *7*, e39939.
- Khakpai, F., Nasehi, M., Haeri-Rohani, A., Eidi, A., & Zarrindast, M. R. (2012). Scopolamine induced memory impairment; possible involvement of NMDA receptor mechanisms of dorsal hippocampus and/or septum. *Behavioral Brain Research*, *231*, 1-10.
- Kheirbek, M. A., Drew, L. J., Burghardt, N. S., Costantini, D. O., Tannenholz, L., Ahmari, S. E. et al. (2013). Differential Control of Learning and Anxiety along the Dorsoventral Axis of the Dentate Gyrus. *Neuron*, *77*, 955-968.
- Kim, J. H., Liao, D., Lau, L. F., & Huganir, R. L. (1998). SynGAP: a synaptic RasGAP that associates with the PSD-95/SAP90 protein family. *Neuron*, *20*, 683-691.
- Klinkenberg, I., & Blokland, A. (2010). The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. *Neuroscience & Biobehavioural Reviews*, *34*, 1307-1350.
- Krapivinsky, G., Krapivinsky, L., Manasian, Y., Ivanov, A., Tyzio, R., Pellegrino, C. et al. (2003). The NMDA receptor is coupled to the ERK pathway by a direct interaction between NR2B and RasGRF1. *Neuron*, *40*, 775-784.
- Kwak, S. P., Hakes, D. J., Martell, K. J., & Dixon, J. E. (1994). Isolation and Characterization of A Human Dual-Specificity Protein-Tyrosine-Phosphatase Gene. *Journal of Biological Chemistry*, *269*, 3596-3604.
- Lacroix L., White I., & Feldon, J. (2002) Effect of excitotoxic lesions of rat medial prefrontal cortex on spatial memory. *Behavioral Brain Research*, *133*, 69-81.
- Lafay-Chebassier, C., Perault-Pochat, M. C., Page, G., Bilan, A. R., Damjanac, M., Pain, S. et al. (2006). The immunosuppressant rapamycin exacerbates neurotoxicity of A beta peptide. *Journal of Neuroscience Research*, *84*, 1323-1334.
- Lana, D., Cerbai, F., Di Russo, J., Boscaro, F., Giannetti, A., Petkova-Kirova, P. et al. (2013). Hippocampal long term memory: Effect of the cholinergic system on local protein synthesis. *Neurobiology of Learning and Memory*, *106*, 246-257.
- Lecourtier, L., de Vasconcelos, A. P., Leroux, E., Cosquer, B., Geiger, K., Lithfous, S. et al. (2011). Septohippocampal pathways contribute to system consolidation of a spatial memory: Sequential implication of gabaergic and cholinergic neurons. *Hippocampus*, *21*, 1277-1289.
- Lena, C., Changeux, J. P., & Mulle, C. (1993). Evidence for “preterminal” nicotinic receptors on GABAergic axons in the rat interpeduncular nucleus. *Journal of Neuroscience*, *13*, 2680–2688.
- Lev, S., Moreno, H., Martinez, R., Canoll, P., Peles, E., Musacchio, J. M. et al. (1995). Protein tyrosine kinase PYK2 involved in Ca(2+)-induced regulation of ion channel and MAP kinase functions. *Nature*, *376*, 737-745.

- Levenson, J. M., O'Riordan, K. J., Brown, K. D., Trinh, M. A., Molfese, D. L., & Sweatt, J. D. (2004). Regulation of histone acetylation during memory formation in the hippocampus. *Journal of Biological Chemistry*, 279, 40545-40559.
- Levey, A. I., Edmunds, S. M., Hersch, S. M., Wiley, R. G., & Heilman, C. J. (1995). Light and electron microscopic study of m2 muscarinic acetylcholine receptor in the basal forebrain of the rat. *Journal of Comparative Neurology*, 351, 339-356.
- Levin, E. D., Bradley, A., Addy, N., & Sigurani, N. (2002). Hippocampal alpha 7 and alpha 4 beta 2 nicotinic receptors and working memory. *Neuroscience*, 109, 757-765.
- Levin, E. D., McClellon, F. J., & Rezvani, A. H. (2006). Nicotinic effects on cognitive function: behavioral characterization, pharmacological specification, and anatomic localization. *Psychopharmacology*, 184, 523-539.
- Lisman, J. E., & Fallon, J. R. (1999). Neuroscience - What maintains memories? *Science*, 283, 339-340.
- Lisman, J. E., & Grace, A. A. (2005). The hippocampal-VTA loop: controlling the entry of information into long-term memory. *Neuron*, 46, 703-713.
- Ma, T., Hoeffler, C. A., Capetillo-Zarate, E., Yu, F., Wong, H., Lin, M. T. et al. (2010). Dysregulation of the mTOR pathway mediates impairment of synaptic plasticity in a mouse model of Alzheimer's disease. *PLoS One.*, 5, e12945.
- Madison, D. V., Lancaster, B., & Nicoll, R. A. (1987). Voltage clamp analysis of cholinergic action in the hippocampus. *Journal of Neuroscience*, 7, 733-741.
- Maren, S. (2001). Is there savings for pavlovian fear conditioning after neurotoxic basolateral amygdala lesions in rats? *Neurobiology of Learning and Memory*, 76, 268-283.
- Margiotta, J. F., Berg, D. K., & Dionne, V. E. (1987). Cyclic AMP regulates the proportion of functional acetylcholine receptors on chicken ciliary ganglion neurons. *Proceedings of the National Academy of Sciences of the United States of America*, 84, 8155-8159
- Marino, M. J., Rouse, S. T., Levey, A. I., Potter, L. T., & Conn, P. J. (1998). Activation of the genetically defined m1 muscarinic receptor potentiates N-methyl-D-aspartate(NMDA) receptor currents in hippocampal pyramidal cells. *Proceedings of the National Academy of Sciences of the United States of America*, 95, 11465-11470.
- Markram, H., & Segal, M. (1990). Long-lasting facilitation of excitatory postsynaptic potentials in the rat hippocampus by acetylcholine. *The Journal of Physiology*, 427, 381-393.
- Marshall, C. J. (1995). Specificity of receptor tyrosine kinase signaling: transient versus sustained extracellular signal-regulated kinase activation. *Cell*, 80, 179-185.
- Marti, B. D., Ramirez, M. R., Dos Reis, E. A., & Izquierdo, I. (2004). Participation of hippocampal nicotinic receptors in acquisition, consolidation and retrieval of memory for one trial inhibitory avoidance in rats. *Neuroscience*, 126, 651-656.
- Martin, D. E., & Hall, M. N. (2005). The expanding TOR signaling network. *Current Opinion in Cell Biology*, 17, 158-166.

- Mazzucchelli, C., Vantaggiato, C., Ciamei, A., Fasano, S., Pakhotin, P., Krezel, W. et al. (2002). Knockout of ERK1 MAP kinase enhances synaptic plasticity in the striatum and facilitates striatal-mediated learning and memory. *Neuron*, *34*, 807-820.
- McGaugh, J. L. (1966). Time-dependent processes in memory storage. *Science*, *153*, 1351-1358.
- McGaugh, J. L., & Izquierdo, I. (2000). The contribution of pharmacology to research on the mechanisms of memory formation. *Trends in Pharmacological Sciences*, *21*, 208-210.
- McGehee, D. S., Heath, M. J., Gelber, S., Devay, P., & Role, L. W. (1995). Nicotine enhancement of fast excitatory synaptic transmission in CNS by presynaptic receptors. *Science*, *269*, 1692-1696.
- McQuiston, A. R., & Madison, D. V. (1999). Nicotinic receptor activation excites distinct subtypes of interneurons in the rat hippocampus. *Journal of Neuroscience*, *19*, 2887-2896.
- Mesulam, M. M., Mufson, E. J., Vainer, B. H., & Levey, A. I. (1983). Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). *Neuroscience*, *10*, 1185-1201.
- Metherate, R. (1998). Synaptic mechanisms in auditory cortex function. *Frontiers in Bioscience*, *3*, d494-d501.
- Misra-Press, A., Rim, C. S., Yao, H., Roberson, M. S., & Stork, P. J. (1995). A novel mitogen-activated protein kinase phosphatase. Structure, expression, and regulation. *Journal of Biological Chemistry*, *270*, 14587-14596.
- Mitsushima, D., Sano, A., & Takahashi, T. (2013). A cholinergic trigger drives learning-induced plasticity at hippocampal synapses. *Nature Communications*, *4*, 2760-2769.
- Morozov, A., Muzzio, I. A., Bourtchouladze, R., Van Strien, N., Lapidus, K., Yin, D. et al. (2003). Rap1 couples cAMP signaling to a distinct pool of p42/44MAPK regulating excitability, synaptic plasticity, learning, and memory. *Neuron*, *39*, 309-325.
- Muda, M., Theodosiou, A., Rodrigues, N., Boschert, U., Camps, M., Gillieron, C. et al. (1996). The dual specificity phosphatases M3/6 and MKP-3 are highly selective for inactivation of distinct mitogen-activated protein kinases. *Journal of Biological Chemistry*, *271*, 27205-27208.
- Muir, J. L., Everitt, B. J., & Robbins, T. W. (1996). The cerebral cortex of the rat and visual attentional function: dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. *Cerebral Cortex*, *6*, 470-481.
- Mulle, C., Choquet, D., Korn, H., & Changeux, J. P. (1992). Calcium influx through nicotinic receptor in rat central neurons: its relevance to cellular regulation. *Neuron*, *8*, 135-143.
- Muthalif, M. M., Benter, I. F., Uddin, M. R., & Malik, K. U. (1996). Calcium/calmodulin-dependent protein kinase II α mediates activation of mitogen-activated protein kinase and cytosolic phospholipase A2 in norepinephrine-induced arachidonic acid release in rabbit aortic smooth muscle cells. *Journal of Biological Chemistry*, *271*, 30149-30157.

- Myskiw, J. C., Rossato, J. I., Bevilaqua, L. R., Medina, J. H., Izquierdo, I., & Cammarota, M. (2008). On the participation of mTOR in recognition memory. *Neurobiology of Learning and Memory*, *89*, 338-351.
- Nomura, Y., Nishiyama, N., Saito, H., & Matsuki, N. (1994) Role of cholinergic neurotransmission in the amygdala on performances of passive avoidance learning in mice *Biological & Pharmaceutical Bulletin*, *17*, 490-494.
- Ohno, M., Yamamoto, T., & Watanabe, S. (1993). Blockade of Hippocampal Nicotinic Receptors Impairs Working Memory But Not Reference Memory in Rats. *Pharmacology Biochemistry and Behavior*, *45*, 89-93.
- Ohno, M., & Watanabe, S. (1996). D-cycloserine, a glycine site agonist, reverses working memory failure by hippocampal muscarinic receptor blockade in rats. *European Journal of Pharmacology*, *318*, 267-271.
- Origlia, N., Kuczewski, N., Aztiria, E., Gautam, D., Wess, J., & Domenici, L. (2006). Muscarinic acetylcholine receptor knockout mice show distinct synaptic plasticity impairments in the visual cortex. *Journal of Physiology*, *577*, 829-840.
- Orr, P.T., Rubin, A.J., Fan, L., Kent, B.A., & Frick, K.M. (2012). The progesterone-induced enhancement of object recognition memory consolidation involves activation of the extracellular signal-regulated kinase (ERK) and mammalian target of rapamycin (mTOR) pathways in the dorsal hippocampus. *Hormones and Behavior*, *61*, 487-495.
- Panja, D., Dageyte, G., Bidinosti, M., Wibrand, K., Kristiansen, A. M., Sonenberg, N. et al. (2009). Novel Translational Control in Arc-dependent Long Term Potentiation Consolidation in Vivo. *Journal of Biological Chemistry*, *284*, 31498-31511.
- Parsons, R. G., Gafford, G. M., & Helmstetter, F. J. (2006). Translational control via the mammalian target of rapamycin pathway is critical for the formation and stability of long-term fear memory in amygdala neurons. *Journal of Neuroscience*, *26*, 12977-12983.
- Paul, S., Nairn, A. C., Wang, P., & Lombroso, P. J. (2003). NMDA-mediated activation of the tyrosine phosphatase STEP regulates the duration of ERK signaling. *Nature Neuroscience*, *6*, 34-42.
- Pazzagli, A., & Pepeu, G. (1964). Amnesic properties of scopolamine and brain acetylcholine in the rat. *International Journal of Pharmacology*, *4*, 291-299.
- Peavy, R. D., & Conn, P. J. (1998). Phosphorylation of mitogen-activated protein kinase in cultured rat cortical glia by stimulation of metabotropic glutamate receptors. *Journal of Neurochemistry*, *71*, 603-612.
- Pepeu, G., & Giovannini, M. G. (2006). The role of cholinergic system in cognitive processes. In E. Giacobini & G. Pepeu (Eds.), *Brain Cholinergic Mechanisms* (pp. 221–233). Oxford: Taylor & Francis.
- Portugal, G. S., & Gould, T. J. (2009). Nicotine withdrawal disrupts new contextual learning. *Pharmacology Biochemistry and Behavior*, *92*, 117-123.

- Poteet-Smith, C. E., Smith, J. A., Lannigan, D. A., Freed, T. A., & Sturgill, T. W. (1999). Generation of constitutively active p90 ribosomal S6 kinase in vivo. Implications for the mitogen-activated protein kinase-activated protein kinase family. *Journal of Biological Chemistry*, *274*, 22135-22138.
- Pouyssegur, J., Volmat, V., & Lenormand, P. (2002). Fidelity and spatio-temporal control in MAP kinase (ERKs) signalling. *Biochemical Pharmacology*, *64*, 755-763.
- Power A. E., & McGaugh J. L. (2002). Phthalic acid amygdalopetal lesion of the nucleus basalis magnocellularis induces reversible memory deficits in rats. *Neurobiology of Learning & Memory*, *77*, 372-388.
- Power, A. E., Thal, L. J., & McGaugh J. L. (2002). Lesions of the nucleus basalis magnocellularis induced by 192 IgG-saporin block memory enhancement with posttraining norepinephrine in the basolateral amygdala. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 2315–2319.
- Power, J. M., & Sah, P. (2002). Nuclear calcium signaling evoked by cholinergic stimulation in hippocampal CA1 pyramidal neurons. *Journal of Neuroscience*, *22*, 3454–3462.
- Puighermanal, E., Marsicano, G., Busquets-Garcia, A., Lutz, B., Maldonado, R., & Ozaita, A. (2009). Cannabinoid modulation of hippocampal long-term memory is mediated by mTOR signaling. *Nature Neuroscience*, *12*, 1152-1158.
- Quevedo, J., Vianna, M. R., Roesler, R., de Paris, F., Izquierdo, I., & Rose, S. P. (1999). Two time windows of anisomycin-induced amnesia for inhibitory avoidance training in rats: protection from amnesia by pretraining but not pre-exposure to the task apparatus. *Learning & Memory*, *6*, 600-607.
- Quirarte, G. L, Cruz-Morales, S. E., Cepeda, A., Garcia-Montañez, M., Roldán-Roldán, G., & Prado-Alcalá, R. A. (1994). Effects of central muscarinic blockade on passive avoidance: anterograde amnesia, state dependency, or both? *Behavioral and Neural Biology*, *62*, 15–20.
- Quirion, R., Wilson, A., Rowe, W., Aubert, I., Richard, J., Doods, H. et al. (1995). Facilitation of acetylcholine release and cognitive performance by an M2-muscarinic receptor antagonist in aged memory-impaired rats. *Journal of Neuroscience*, *15*, 1455-1462.
- Ragozzino, M. E., Pal, S. N., Unick, K., Stefani, M. R., & Gold, P. E. (1998). Modulation of hippocampal acetylcholine release and spontaneous alternation scores by intrahippocampal glucose injections. *Journal of Neuroscience*, *18*, 1595-1601.
- Raiteri, M., Leardi, R., & Marchi, M. (1984). Heterogeneity of presynaptic muscarinic receptors regulating neurotransmitter release in the rat brain. *Journal of Pharmacology and Experimental Therapeutics*, *228*, 209-214.
- Rastogi, S., Unni, S., Sharma, S., Rao Laxmi, T., & Kutty, B.M. (2014) Cholinergic immunotoxin 192 IgG-SAPORIN alters subicular theta–gamma activity and impairs spatial learning in rats, *Neurobiology of Learning and Memory*, *114*, 117–126.

- Raybuck, J. D., & Gould, T. J. (2007). Extracellular signal-regulated kinase 1/2 involvement in the enhancement of contextual fear conditioning by nicotine. *Behavioral Neuroscience*, *121*, 1119-1124.
- Roland, J. J., Stewart, A. L., Janke, K. L., Gielow, M. R., Kostek, J. A., Savage, L. M. et al. (2014). Medial Septum-Diagonal Band of Broca (MSDB) GABAergic Regulation of Hippocampal Acetylcholine Efflux Is Dependent on Cognitive Demands. *Journal of Neuroscience*, *34*, 506-514.
- Roldán, G., Bolaños-Badillo, E., González-Sánchez, H., Quirarte, G. L., & Prado-Alcalá, R. A. (1997). Selective M1 muscarinic receptor antagonists disrupt memory consolidation of inhibitory avoidance in rats. *Neurosci Letters*, *230*, 93-96.
- Rosenblum, K., Futter, M., Jones, M., Hulme, E. C., & Bliss, T. V. (2000). ERK1/II regulation by the muscarinic acetylcholine receptors in neurons. *Journal of Neuroscience*, *20*, 977-985.
- Rush, D. K. (1988). Scopolamine amnesia of passive avoidance: a deficit of information acquisition. *Behavioral and Neural Biology*, *50*, 255-274.
- Russo, C., Marchi, M., Andrioli, G. C., Cavazzani, P., & Raiteri, M. (1993). Enhancement of Glycine Release from Human Brain Cortex Synaptosomes by Acetylcholine Acting at M(4)-Muscarinic Receptors. *Journal of Pharmacology and Experimental Therapeutics*, *266*, 142-146.
- Saba-El-Leil, M. K., Vella, F. D., Vernay, B., Voisin, L., Chen, L., Labrecque, N. et al. (2003). An essential function of the mitogen-activated protein kinase Erk2 in mouse trophoblast development. *EMBO Reports*, *4*, 964-968.
- Sargent, P. B. (1993). The diversity of neuronal nicotinic acetylcholine receptors. *Annual Reviews in Neuroscience*, *16*, 403-443.
- Sarter, M., & Bruno, J. P. (1997a). Cognitive functions of cortical acetylcholine: toward a unifying hypothesis. *Brain Research Reviews*, *23*, 28-46.
- Sarter, M., & Bruno, J. P. (1997b). Trans-synaptic stimulation of cortical acetylcholine and enhancement of attentional functions: a rational approach for the development of cognition enhancers. *Behavioral Brain Research*, *83*, 7-14.
- Sarter, M., & Bruno, J. P. (2000). Cortical cholinergic inputs mediating arousal, attentional processing and dreaming: differential afferent regulation of the basal forebrain by telencephalic and brainstem afferents. *Neuroscience*, *95*, 933-952.
- Savage, U. C., Faust, W. B., Lambert, P., & Moerschbaeche, J. M. (1996). Effects of scopolamine on learning and memory in monkeys. *Psychopharmacology (Berl)*, *123*, 9-14.
- Scali, C., Vannucchi, M. G., Pepeu, G., & Casamenti, F. (1995). Peripherally injected scopolamine differentially modulates acetylcholine release in vivo in the young and aged rats. *Neuroscience Letters*, *197*, 171-174.
- Schafe, G. E., Atkins, C. M., Swank, M. W., Bauer, E. P., Sweatt, J. D., & LeDoux, J. E. (2000). Activation of ERK/MAP kinase in the amygdala is required for memory consolidation of pavlovian fear conditioning. *Journal of Neuroscience*, *20*, 8177-8187.

- Schafe, G. E., Nadel, N. V., Sullivan, G. M., Harris, A., & LeDoux, J. E. (1999). Memory consolidation for contextual and auditory fear conditioning is dependent on protein synthesis, PKA, and MAP kinase. *Learning & Memory*, 6, 97-110.
- Schicknick, H., Schott, B. H., Budinger, E., Smalla, K. H., Riedel, A., Seidenbecher, C. I. et al. (2008). Dopaminergic Modulation of Auditory Cortex-Dependent Memory Consolidation through mTOR. *Cerebral Cortex*, 18, 2646-2658.
- Seger, R., & Krebs, E. G. (1995). The MAPK signaling cascade. *FASEB Journal*, 9, 726-735.
- Selcher, J. C., Nekrasova, T., Paylor, R., Landreth, G. E., & Sweatt, J. D. (2001). Mice lacking the ERK1 isoform of MAP kinase are unimpaired in emotional learning. *Learning & Memory*, 8, 11-19.
- Sharma, G., & Vijayaraghavan, S. (2003). Modulation of presynaptic store calcium induces release of glutamate and postsynaptic firing. *Neuron* 38, 929-939.
- Sharrocks, A. D., Yang, S. H., & Galanis, A. (2000). Docking domains and substrate-specificity determination for MAP kinases. *Trends in Biochemical Sciences*, 25, 448-453.
- Slack, B. E., & Blusztajn, J. K. (2008). Differential regulation of mTOR-dependent S6 phosphorylation by muscarinic acetylcholine receptor subtypes. *Journal of Cellular Biochemistry*, 104, 1818-1831.
- Slipczuk, L., Bekinschtein, P., Katche, C., Cammarota, M., Izquierdo, I., & Medina, J. H. (2009). BDNF activates mTOR to regulate GluR1 expression required for memory formation. *PLoS. One*, 4(6), e6007.
- Squire, L. R. (1992). Memory and the hippocampus: a synthesis from findings with rats, monkeys, and humans. *Psychological Review*, 99, 195-231.
- St. Augustine 398. *Confessions - Book X*. Translated by Edward Bouverie Pusey.
- Stancampiano, R., Cocco, S., Cugusi, C., Sarais, L., & Fadda, F. (1999). Serotonin and acetylcholine release response in the rat hippocampus during a spatial memory task. *Neuroscience*, 89, 1135-1143.
- Stanhope, K. J., McLenachan, A. P., & Dourish, C. T. (1995). Dissociation between cognitive and motor/motivational deficits in the delayed matching to position test: effects of scopolamine, 8-OH-DPAT and EAA antagonists. *Psychopharmacology (Berl)*, 122, 268-280.
- Subramaniam, S., Zirrgiebel, U., Bohlen Und, H. O., Strelau, J., Laliberte, C., Kaplan, D. R. et al. (2004). ERK activation promotes neuronal degeneration predominantly through plasma membrane damage and independently of caspase-3. *Journal of Cell Biology*, 165, 357-369.
- Sudweeks, S. N., & Yakel, J. L. (2000). Functional and molecular characterization of neuronal nicotinic ACh receptors in rat CA1 hippocampal neurons. *Journal of Physiology-London*, 527, 515-528.
- Sui, L., Wang, J., & Li, B. M. (2008). Role of the phosphoinositide 3-kinase-Akt-mammalian target of the rapamycin signaling pathway in long-term potentiation and trace fear conditioning memory in rat medial prefrontal cortex. *Learning & Memory*, 15, 762-776.

- Sun, X., Ritzenthaler, J. D., Zhong, X., Zheng, Y., Roman, J., & Han, S. (2009). Nicotine stimulates PPARbeta/delta expression in human lung carcinoma cells through activation of PI3K/mTOR and suppression of AP-2alpha. *Cancer Research*, *69*, 6445-6453.
- Sweatt, J. D. (2001). The neuronal MAP kinase cascade: a biochemical signal integration system subserving synaptic plasticity and memory. *Journal of Neurochemistry*, *76*, 1-10.
- Sweatt, J. D. (2004). Mitogen-activated protein kinases in synaptic plasticity and memory. *Current Opinion in Neurobiology*, *14*, 311-317.
- Szabo, G. G., Holderith, N., Gulyas, A. I., Freund, T. F., & Hajos, N. (2010). Distinct synaptic properties of perisomatic inhibitory cell types and their different modulation by cholinergic receptor activation in the CA3 region of the mouse hippocampus. *European Journal of Neuroscience*, *31*, 2234–2246.
- Takei, N., Inamura, N., Kawamura, M., Namba, H., Hara, K., Yonezawa, K. et al. (2004). Brain-derived neurotrophic factor induces mammalian target of rapamycin-dependent local activation of translation machinery and protein synthesis in neuronal dendrites. *Journal of Neuroscience*, *24*, 9760-9769.
- Takei, N., Kawamura, M., Hara, K., Yonezawa, K., & Nawa, H. (2001). Brain-derived neurotrophic factor enhances neuronal translation by activating multiple initiation processes - Comparison with the effects of insulin. *Journal of Biological Chemistry*, *276*, 42818-42825.
- Tang, A. H., Karson, M. A., Nagode, D. A., McIntosh, J. M., Uebele, V. N., Renger, J. J., et al. (2011). Nerve terminal nicotinic acetylcholine receptors initiate quantal GABA release from perisomatic interneurons by activating axonal T-type (Cav3) Ca²⁺ channels and Ca²⁺ release from stores. *Journal of Neuroscience*, *31*, 13546–13561.
- Teles-Griolo Ruivo LM, & Mellor JR (2013). Cholinergic modulation of hippocampal network function. *Frontiers in Synaptic Neurosciences*, *5*, 1-15.
- Tischmeyer, W., Schicknick, H., Kraus, M., Seidenbecher, C. I., Staak, S., Scheich, H. et al. (2003). Rapamycin-sensitive signalling in long-term consolidation of auditory cortex-dependent memory. *European Journal of Neuroscience*, *18*, 942-950.
- Tiunova, A., Anokhin, K., Rose, S. P., & Mileusnic, R. (1996). Involvement of glutamate receptors, protein kinases, and protein synthesis in memory for visual discrimination in the young chick. *Neurobiology of Learning and Memory*, *65*, 233-234.
- Torres, E. M., Perry, T. A., Blockland, A., Wilkinson, L. S., Wiley, R. G., Lappi D. A., et al. (1994). Behavioural, histochemical and biochemical consequences of selective immunolesions in discrete regions of the basal forebrain cholinergic system. *Neuroscience*, *63*, 95-122.
- Toselli, M., Lang, J., Costa, T., & Lux, H. D. (1989). Direct modulation of voltage-dependent calcium channels by muscarinic activation of a pertussis toxin-sensitive G-protein in hippocampal neurons. *Pflugers Archives: European Journal of Physiology*, *415*, 255–261.

- Tranel, D., Damasio, A.R. (1995) Neurobiological foundations of human memory. In A.D. Baddely, B.A. Wilson & F.N Watts (Eds), *Handbook of memory disorders* (pp 27 - 50), Chichester: John Wiley & Sons.
- Traverse, S., Gomez, N., Paterson, H., Marshall, C., & Cohen, P. (1992). Sustained activation of the mitogen-activated protein (MAP) kinase cascade may be required for differentiation of PC12 cells. Comparison of the effects of nerve growth factor and epidermal growth factor. *Biochemical Journal*, 288, 351-355.
- Treisman, R. (1995). Journey to the surface of the cell: Fos regulation and the SRE. *EMBO Journal*, 14, 4905-4913.
- Treisman, R. (1996). Regulation of transcription by MAP kinase cascades. *Current Opinion in Cell Biology*, 8, 205-215.
- Tremblay, N., Warren, R. A., & Dykes, R. W. (1990). Electrophysiological studies of acetylcholine and the role of the basal forebrain in the somatosensory cortex of the cat. II. Cortical neurons excited by somatic stimuli. *Journal of Neurophysiology*, 64, 1212-1222.
- Tsokas, P., Ma, T., Iyengar, R., Landau, E. M., & Blitzler, R. D. (2007). Mitogen-activated protein kinase upregulates the dendritic translation machinery in long-term potentiation by controlling the mammalian target of rapamycin pathway. *Journal of Neuroscience*, 27, 5885-5894.
- Vannucchi, M. G., & Pepeu, G. (1995). Muscarinic receptor modulation of acetylcholine release from rat cerebral cortex and hippocampus. *Neuroscience Letters*, 190, 53-56.
- Vannucchi, M. G., Scali, C., Kopf, S. R., Pepeu, G., & Casamenti, F. (1997). Selective muscarinic antagonists differentially affect in vivo acetylcholine release and memory performances of young and aged rats. *Neuroscience*, 79, 837-846.
- Vernino, S., Amador, M., Luetje, C. W., Patrick, J., & Dani, J. A. (1992). Calcium modulation and high calcium permeability of neuronal nicotinic acetylcholine receptors. *Neuron*, 8, 127-134.
- Vianna, M. R., Izquierdo, L. A., Barros, D. M., Ardenghi, P., Pereira, P., Rodrigues, C. et al. (2000). Differential role of hippocampal cAMP-dependent protein kinase in short- and long-term memory. *Neurochemical Research*, 25, 621-626.
- Vianna, M. R., Izquierdo, L. A., Barros, D. M., Medina, J. H., & Izquierdo, I. (1999). Intrahippocampal infusion of an inhibitor of protein kinase A separates short- from long-term memory. *Behavioural Pharmacology*, 10, 223-227.
- Vijayaraghavan, S., Pugh, P. C., Zhang, Z. W., Rathouz, M. M., & Berg, D.K. (1992). Nicotinic receptors that bind alpha-bungarotoxin on neurons raise intracellular free Ca²⁺. *Neuron*, 8, 353-362.
- Vinogradova, O. S. (2001). Hippocampus as comparator: role of the two input and two output systems of the hippocampus in selection and registration of information. *Hippocampus*, 11, 578-598.

- Waite, J. J., & Thal, L. J. (1996). Lesions of the cholinergic nuclei in the rat basal forebrain: Excitotoxins vs an immunotoxin. *Life Sciences*, *58*, 1947-1953.
- Walters, C. L., Cleck, J. N., Kuo, Y. C., & Blendy, J. A. (2005). mu-Opioid receptor and CREB activation are required for nicotine reward. *Neuron*, *46*, 933-943.
- Walz, R., Roesler, R., Quevedo, J., Rockenbach, I. C., Amaral, O. B., Vianna, M. R. et al. (1999). Dose-dependent impairment of inhibitory avoidance retention in rats by immediate post-training infusion of a mitogen-activated protein kinase kinase inhibitor into cortical structures. *Behavioral Brain Research*, *105*, 219-223.
- Walz, R., Roesler, R., Quevedo, J., Sant'Anna, M. K., Madruga, M., Rodrigues, C. et al. (2000). Time-dependent impairment of inhibitory avoidance retention in rats by posttraining infusion of a mitogen-activated protein kinase kinase inhibitor into cortical and limbic structures. *Neurobiology of Learning and Memory*, *73*, 11-20.
- Wanaverbecq, N., Semyanov, A., Pavlov, I., Walker, M. C., & Kullmann, D. M. (2007). Cholinergic axons modulate GABAergic signaling among hippocampal interneurons via postsynaptic alpha7 nicotinic receptors. *Journal of Neuroscience*, *27*, 5683-5693.
- Wang, L., & Proud, C. G. (2002). Regulation of the phosphorylation of elongation factor 2 by MEK-dependent signalling in adult rat cardiomyocytes. *FEBS Letters*, *531*, 285-289.
- Watabe, A. M., Zaki, P. A., & O'Dell, T. J. (2000). Coactivation of beta-adrenergic and cholinergic receptors enhances the induction of long-term potentiation and synergistically activates mitogen-activated protein kinase in the hippocampal CA1 region. *Journal of Neuroscience*, *20*, 5924-5931.
- Watanabe, S., Hoffman, D. A., Migliore, M., & Johnston, D. (2002). Dendritic K⁺ channels contribute to spike-timing dependent long-term potentiation in hippocampal pyramidal neurons. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 8366-8371.
- Wiener, N. I., & Messer J. (1973). Scopolamine-induced impairment of long-term retention in rats. *Behavioral Biology*, *9*, 227-234
- Wilensky, A. E., Schafe, G. E., & LeDoux, J. E. (2000). The amygdala modulates memory consolidation of fear-motivated inhibitory avoidance learning but not classical fear conditioning. *Journal of Neuroscience*, *20*, 7059-7066.
- Wiley, R.G., Oeltmann, T.N., & Lappi, D.A. (1991). Immunolesioning: Selective destruction of neurons using immunotoxin to rat NGF receptor. *Brain Research*, *562*:149-153.
- Wilkinson, J. L., & Bevins, R. A. (2008). Intravenous nicotine conditions a place preference in rats using an unbiased design. *Pharmacology Biochemistry and Behavior*, *88*, 256-264.
- Williams, N. G., Zhong, H., & Minneman, K. P. (1998). Differential coupling of alpha1-, alpha2-, and beta-adrenergic receptors to mitogen-activated protein kinase pathways and differentiation in transfected PC12 cells. *Journal of Biological Chemistry*, *273*, 24624-24632.

- Winder, D. G., Martin, K. C., Muzzio, I. A., Rohrer, D., Chruscinski, A., Kobilka, B. et al. (1999). ERK plays a regulatory role in induction of LTP by theta frequency stimulation and its modulation by beta-adrenergic receptors. *Neuron*, *24*, 715-726.
- Wonnacott, S. (1997). Presynaptic nicotinic ACh receptors. *Trends in Neurosciences*, *20*, 92–98.
- Wu, G. Y., Deisseroth, K., & Tsien, R. W. (2001). Spaced stimuli stabilize MAPK pathway activation and its effects on dendritic morphology. *Nature Neuroscience*, *4*, 151-158.
- Xia, Z., Dudek, H., Miranti, C. K., & Greenberg, M. E. (1996). Calcium influx via the NMDA receptor induces immediate early gene transcription by a MAP kinase/ERK-dependent mechanism. *Journal of Neuroscience*, *16*, 5425-5436.
- Xu, J., Weng, Y. I., Simonyi, A., Krugh, B. W., Liao, Z., Weisman, G. A. et al. (2002). Role of PKC and MAPK in cytosolic PLA2 phosphorylation and arachadonic acid release in primary murine astrocytes. *Journal of Neurochemistry*, *83*, 259-270.
- Yanow, S. K., Manseau, F., Hislop, J., Castellucci, V. F., & Sossin, W. S. (1998). Biochemical pathways by which serotonin regulates translation in the nervous system of *Aplysia*. *Journal of Neurochemistry*, *70*, 572-583.
- Yao, Y., Li, W., Wu, J., Germann, U. A., Su, M. S., Kuida, K. et al. (2003). Extracellular signal-regulated kinase 2 is necessary for mesoderm differentiation. *Proceedings of the National Academy of Sciences of the United States of America*, *100*, 12759-12764.
- Yasoshima, Y., & Yamamoto, T. (1997). Rat gustatory memory requires protein kinase C activity in the amygdala and cortical gustatory area. *NeuroReport*, *8*, 1363-1367.
- York, R. D., Yao, H., Dillon, T., Ellig, C. L., Eckert, S. P., McCleskey, E. W. et al. (1998). Rap1 mediates sustained MAP kinase activation induced by nerve growth factor. *Nature*, *392*, 622-626.
- Yuan, L. L., Adams, J. P., Swank, M., Sweatt, J. D., & Johnston, D. (2002). Protein kinase modulation of dendritic K⁺ channels in hippocampus involves a mitogen-activated protein kinase pathway. *Journal of Neuroscience*, *22*, 4860-4868.
- Zarrindas, M. R., Sadegh, M., & Shafaghi, B. (1996). Effects of nicotine on memory retrieval in mice. *European Journal of Pharmacology*, *295*, 1-6.
- Zhang, W., Basile, A., Gomeza, J., Volpicelli, L., Levey, A. I., & Wess, J. (2002). Characterization of central inhibitory muscarinic autoreceptors by the use of muscarinic acetylcholine receptor knock-out mice. *Journal of Neuroscience*, *22*, 1709-1717.
- Zheng, Y., Ritzenthaler, J. D., Roman, J., & Han, S. (2007). Nicotine stimulates human lung cancer cell growth by inducing fibronectin expression. *American Journal of Respiratory Cell and Molecular Biology*, *37*, 681-690.
- Zhu, J. J., Qin, Y., Zhao, M., Van Aelst, L., & Malinow, R. (2002). Ras and Rap control AMPA receptor trafficking during synaptic plasticity. *Cell*, *110*, 443-455.

Zola-Morgan, S., & Squire, L. R. (1993). Neuroanatomy of memory. *Annual Reviews in the Neurosciences*, 16, 547-563.

Figure 1

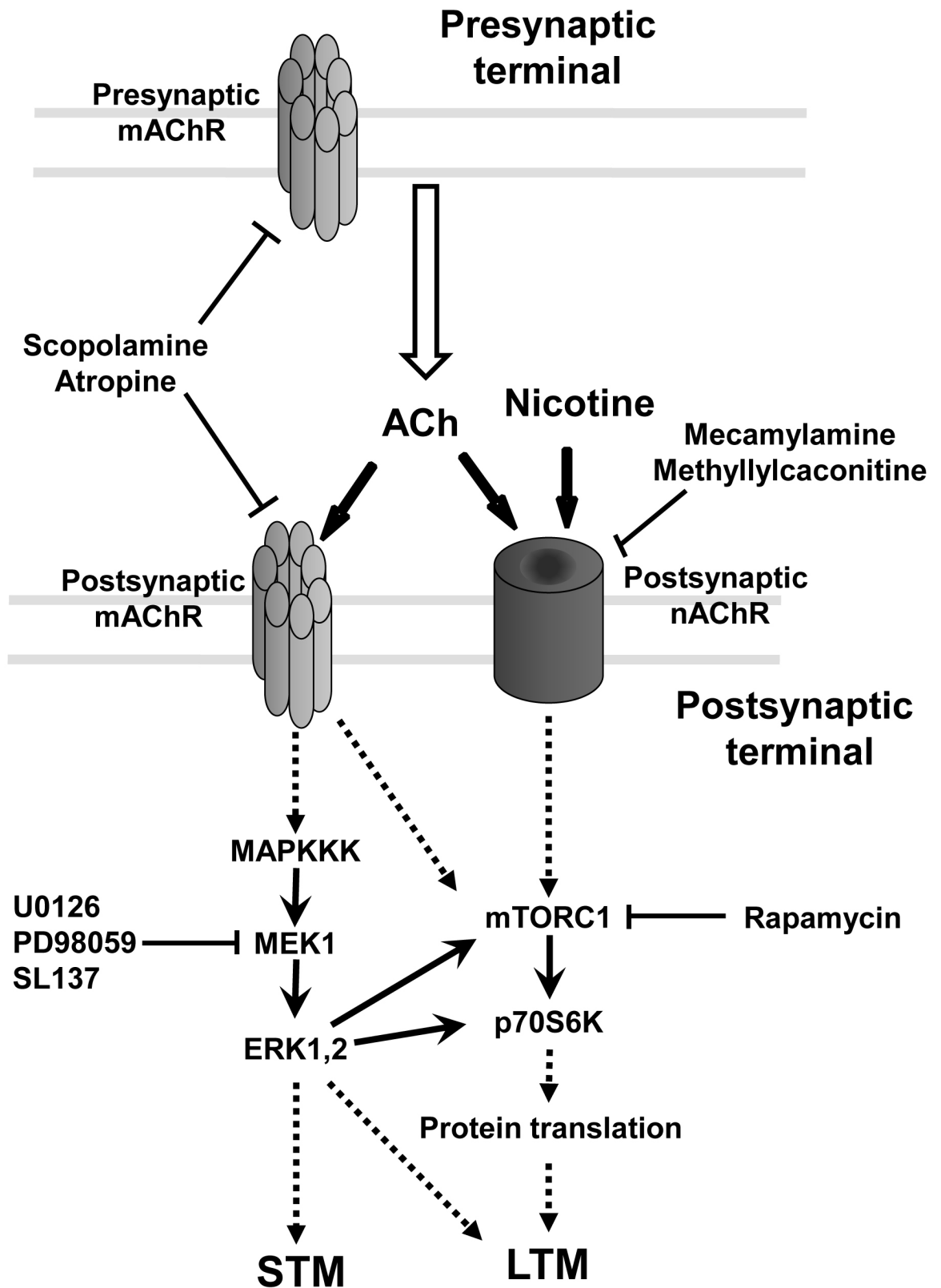


Table 1. *Effect of systemic or local administration of scopolamine on IA in rats and mice*

Inhibitory Avoidance	Species	Scopolamine Effective Doses	Route and time of administration	Effect	References
Step-through	Wistar Rats	8.0 mg, 16 mg	IP, posttraining	Impaired Recall at 24 h	(1)
Step-through	Wistar Rats	0.2 mg	IP, 30 min before training	Impaired Recall at 24 h	(2)
Step-through	Wistar Rats	1.0 mg	IP, 20 min before training	Impaired Recall at 24 h	(3)
Step-through	Wistar Rats	8.0 mg, 12 mg	IP, 5 min before training, Low footshock intensity IP, both 5 min before training and 5 min before recall, High footshock intensity	Impaired Recall Recall not changed (state-dependency)	(4)
Step-through	Wistar Rats	8.0 mg	IP, 5 min after training, Low footshock intensity	Impaired Recall at 24 h	(5)
Step-through	Mice (Std-ddY)	1.0 mg, 2.0 mg	IP, 30 min before training IP, immediately after training IP, 30 min before recall trial	Impaired Recall at 24 h Recall at 24h not changed Recall at 24h not changed	(6)
Step through	Wistar Rats	0.2 mg	SC, 30 min before training	Impaired recall (24h)	(7)
Step down	Wistar Rats	1.5 mg 1.5 mg 3 µg in 1µl	IP, 30 min before training IP, 30 min before recall Intrahippocampus (bilaterally)	Impaired recall (1 h) No effect (1 h) Impaired recall (1 h)	(8)
Step-down	Mice (Std-ddY)	0.5 mg, 1.0 mg 2.0 mg	IP, 30 min before training IP, immediately after training IP, 30 min before recall trial	Impaired Recall at 24 h Recall at 24h not changed Recall at 24h not changed	(6)
Step-through	Mice (NMRI)	0.3 mg, 3.0 mg 30 mg	IP, 5 min before training IP, immediately after training IP, immediately after training	Impaired Recall at 24 h Recall at 24 h not changed Impaired Recall at 24 h	(9)
Step down	Wistar Rats	0.095 mg in 3 µl/side	Intrahippocampus (bilaterally)	Impaired Recall 5, 7 or 10 days but not 1 or 3 days after training	(10)
Step through	Wistar Rats	5-20 µg/rat	ICV, after training	Impaired Recall at 24 h	(11)

References: (1) Roldán, Bolaños-Badillo, González-Sánchez, Quirarte, & Prado-Alcalá (1997); (2) Vannucchi, Scali, Kopf, Pepeu, & Casamenti (1997); (3) Ohno, & Watanabe (1996); (4) Quirarte et al. (1994); (5) Cruz-Morales, Duran-Arevalo, Diaz Del Guante, Quirarte, & Prado-Alcalá, (1992); (6) Nomura, Nishiyama, Saito, & Matsuki (1994); (7) Giovannini, Bartolini, Bacciottini, Greco, & Blandina (1999); (8) Giovannini et al., (2005); (9) Rush (1988); (10) Wiener & Messer (1973); (11) Eidi, Zarrindast, Eidi, Oryan, & Parivar (2003)