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Regulation of the integration of newly generated neurons in the adult hippocampus

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Regulation of the integration of newly generated neurons in the adult hippocampus

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English abstract: Regulation of the integration of newly generated neurons in the adult hippocampus

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Hippocampal adult neurogenesis results in the continuous formation of new neurons in the adult hippocampus, which participate to learning and memory. Manipulations increasing adult neurogenesis have a huge clinical potential in pathologies involving memory loss. Intringuingly, most of the newborn neurons die during their maturation. Thus, increasing newborn neuron survival during their maturation may be a powerful way to increase overall adult neurogenesis. The factors governing this neuronal death are yet poorly known.

In my PhD project, we made the hypothesis that synaptogenesis and synaptic activity play a role in the survival of newborn hippocampal neurons and may therefore lead to increased memory peformances. To this aim, we studied three factors potentially involved in the regulation of the synaptic integration of adult-born neurons.

First, we used propofol anesthesia to provoke a global increase in GABAergic activity of the network, and we evaluated the outcome on newborn neuron synaptic integration, morphological development and survival. Propofol anesthesia impaired the dendritic maturation and survival of adult-born neurons in an age-dependent manner. Next, we examined the development of astrocytic ensheathment on the synapses formed by newborn neurons, as we hypothesized that astrocytes are involved in their synaptic integration. Astrocytic processes ensheathed the synapses of newborn neurons very early in their development, and extracellular glutamate reuptake by their processes modulated synaptic transmission on these cells. Finally, we studied the cell-autonomous effects of the overexpression of synaptic adhesion molecules on the development, synaptic integration and survival of newborn neurons, and we found that manipulating of a single adhesion molecule was sufficient to modify synaptogenesis and/or synapse function, and to modify newborn neuron survival.

Together, these results suggest that the activity of the neuronal network, the modulation of glutamate transport by astrocytes, and the synapse formation and activity of the neuron itself may regulate the survival of newborn neurons. Thus, the survival of newborn neurons may depend on their ability to communicate with the network. This knowledge is crucial for finding ways to increase neurogenesis in patients. More generally, understanding how the neurogenic niche works and which factors are important for the generation, maturation and survival of neurons is fundamental to be able to maybe, one day, replace neurons in any region of the brain

Abstract français : Mécanismes régulant l'intégration synaptique des neurones générés dans l'hippocampe adulte

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De nouveaux neurones sont constamment produits dans l'hippocampe lors de la neurogenèse adulte, et ce processus intervient dans l'apprentissage et la mémoire. Les manipulations augmentant la neurogenèse adulte ont un potentiel énorme en termes d'applications thérapeutiques, dans le cadre de pathologies impliquant une perte de mémoire. Hors, la plupart des neurones générés dans l'hippocampe adulte meurent par apoptose durant leur maturation. Par conséquent, augmenter la survie des nouveaux neurones durant cette période pourrait être un moyen efficace d'augmenter la neurogenèse hippocampale adulte. Les facteurs contrôlant cette mort neuronale sont cependant peu connus.

Dans le cadre de mon projet de thèse, nous avons fait l'hypothèse que la synaptogenèse et l'activité synaptique jouent un rôle dans la survie des nouveaux neurones hippocampaux, et nous avons étudié trois facteurs potentiellement impliqués dans l'intégration synaptique des nouveaux neurones. Dans un premier temps, nous avons utilisé l'anesthésie au propofol pour provoquer une augmentation globale de l'activité GABAergique du réseau neuronal, et nous avons évalué les conséquences de cette anesthésie sur l'intégration synaptique mais aussi sur le développement morphologique et la survie des nouveaux neurones. Nous avons observé une réduction de la maturation dendritique et la survie des nouveaux neurones après anesthésie au propofol, et cet effet dépendait de l'âge des neurones. Nous avons ensuite fait l'hypothèse que les astrocytes étaient impliqués dans l'intégration synaptique des nouveaux neurones, et nous avons examiné le développement des processus astrocytaires autour de leurs synapses. Notre travail a montré que les processus astrocytaires recouvrent les synapses formées par les nouveaux neurones très tôt dans leur développement, et que ces processus modulent la transmission synaptique de ces neurones. Enfin, nous avons étudié les effets directs de la surexpression de molécules d'adhésion dans les nouveaux neurones sur leur développement, leur intégration synaptique et leur survie. Notre étude a montré que la manipulation d'une seule molécule d'adhésion est suffisante pour modifier la synaptogenèse et/ou la fonction synaptique des nouveaux neurones, et pour modifier leur survie.

Ces résultats suggèrent que l'activité du réseau neuronal, la modulation du transport du glutamate par les astrocytes, et la formation et l'activité des synapses du neurone lui-même régulent la survie des nouveaux neurones. Par conséquent, la survie des nouveaux neurones pourrait dépendre de leur capacité à communiquer avec le réseau qui les entoure. Ces connaissances sont fondamentales pour trouver des moyens d'augmenter la neurogenèse adulte chez les patients. De manière plus globale, comprendre les modalités de fonctionnement de la niche neurogénique et découvrir quels sont les facteurs importants dans la genèse, maturation et survie des nouveaux neurones est crucial pour être un jour capable de remplacer des neurones dans d'autres régions du cerveau.

Abbreviations

AD: Alzheimer's disease

AMPA: α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid

APP: Amyloid precursor protein

BLBP: Brain lipid binding protein

BrdU: Bromodeoxyuridine

CA: Cornu Ammonis

CaMKII: CaM kinase II

CFP: Cyan fluorescent protein

CNO: clozapine-N-oxide

CNS: central nervous system

DAPI: 4,6 Diamidino-2-phenylindole

DCX: Doublecortin

DG: Dentate gyrus

DnSynCAM1: Dominant-negative SynCAM1

Dpi: Days post injection

DREADD: Designer Receptor Exclusively Activated by Designer Drugs

dsDNA: Double stranded DNA

EC: Entorhinal cortex

$GABA_AR$: $GABA_A$ receptor

GCL: Granule cell layer

GFAP: Glial Fibrillary Acidic Protein

GFP: Green fluorescent protein

GLAST: Glutamate Aspartate transporter

HD: Huntington's disease

HEK: Human embryonic kidney

KCC2: K^+/Cl^- cotransporter

LTP: Long-term potentiation

MoMuLV: Moloney Murine Leukemia Virus

MSB: Multiple synapse bouton

MSD: Multiple synapse dendrite

MFT: Mossy fiber terminal

NKCC1: $Na^+/K^+/Cl^+$ cotransporter

NL1: Neuroligin-1

NL2: Neuroligin-2

NMDA: N-methyl-D-aspartate

NR1: NMDA receptor 1 subunit

NR2: NMDA receptor 2 subunit

PGE_2: Prostaglandin E2

PSA-NCAM: Polysialylated-neural cell adhesion molecule

PSC: Post-synaptic current

PT: Pseudotetanus protocol

RFP: Red fluorescent protein

SGZ: Subgranular zone

SVZ: Subventricular zone

SSB: Single synapse bouton

SSRI: Serotonin-selective reuptake inhibitor

SynCAM1: Synaptic adhesion molecule 1

VGAT: Vesicular GABA transporter

Introduction

Adult stem cells are undifferentiated cells, found among differentiated cells in a tissue or organ. Adult stem cells can self-renew and can differentiate to yield some or all of the major specialized cell types of the tissue or organ. The primary roles of adult stem cells in a living organism are to maintain and repair the tissue in which they are present.

Research on adult stem cells began in the 1950s, when researchers discovered that bone marrow contains two kinds of stem cells[1]. The first population is called hematopoietic stem cells and forms all the types of blood cells in the body. The second population, called bone marrow stromal stem cells was discovered few years later. These cells can generate bone, cartilage, fat, cells supporting the formation of blood, and fibrous connective tissue.

However, it is only in the 1960s that scientists discovered stem cells in the adult brain, that ultimately become nerve cells [2,3]. The existence of these dividing cells, called adult neural stem cells, was however dismissed for a very long time. In 1983, Nottebohm's group discovered neuronal production in the higher vocal center of the adult female canary brain following systemic testosterone administration. This was later found to be involved in song learning in males [4]. This discovery sparked renewed interest in the topic, but it was not until the 1990s that scientists agreed that the adult mammalian brain contains neural stem cells able to generate the brain's three major cell types: neurons, astrocytes and oligodendrocytes.

These adult neural stem cells continually proliferate, and the process by which they generate neurons is called adult neurogenesis. The neurons generated from adult neural stem cells are called adult-born neurons. This phenomenon occurs in a number of species, including humans and rodents [5-9]. Intriguingly, adult neural stem cells only give rise to neurons in two canonical brain areas: the dentate gyrus of the hippocampus and the subventricular zone. Neuroblasts produced in the subventricular zone (SVZ) then migrate to the olfactory bulb. In adult humans, 700 new neurons are added in each hippocampus per day [8], and in adult rodents, more than 2500 neurons are formed per day in each hippocampus [10]. In contrast, it seems that adult neurogenesis is extremely limited in the human olfactory bulb [11], whereas more than 30000 of neurons are generated and reach the olfactory bulb each day in adult mice [12].

Recent work suggests that adult neurogenesis also takes place in another region, the median eminence of the hypothalamus, but this idea is still debated [13]. Other studies show that, in contrary to rodents, adult-born neurons produced in the human SVZ do not migrate to the

olfactory bulb [11,14]. Interestingly, a study published very recently suggests that in the human, these neurons may migrate to the striatum instead of the olfactory bulb, whereas this has not been described in rodents [15]. Neurogenesis persists at rather high rates during the entire lifespan in humans, at least in the hippocampus, although it declines with age [8,16,17] (Figure 1).

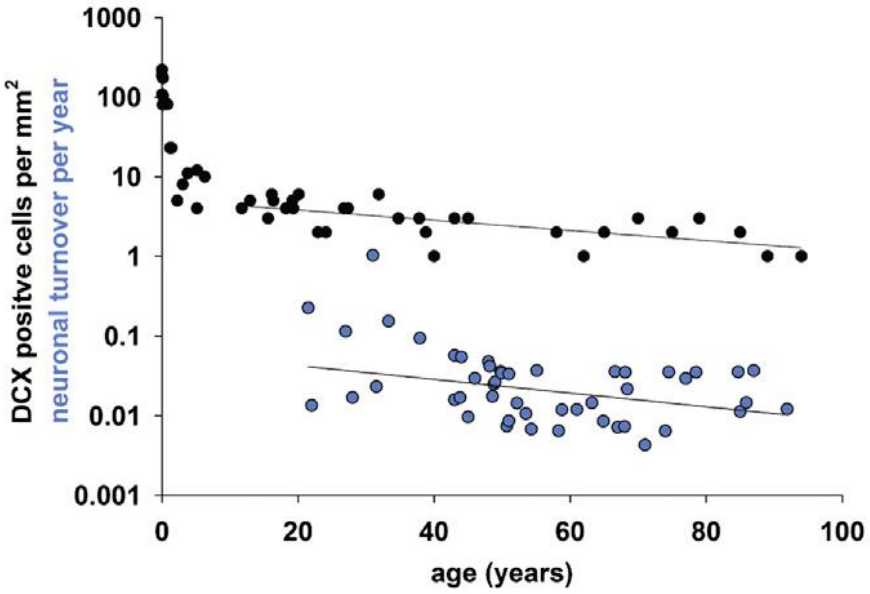


Figure 1: Neuronal Turnover Dynamics in the Human Hippocampus [8]. The number of DCX-positive cells per mm^2 in the human dentate gyrus [17] and the neuronal turnover rates computed from ^{14}C concentration-based modeling show similar modest declines with age during adult ages. Straight lines depict linear regression curves, and the regression line for DCX cell counts was calculated for individuals aged 10 years and older.

The discovery of adult neurogenesis in several regions of the brain raises a number of questions: What is the function of adult-born neurons? How are adult-born generated and how do they integrate into the circuitry? Could they be used as a way to replace damaged or dead cells in the brain?

As the hippocampus is the most important region of neurogenesis in the human brain, and given potential therapeutic applications of adult hippocampal neurogenesis in neurodegenerative diseases such as Alzheimer's disease, this work primarily focuses on the mechanisms of synaptic integration of new neurons in the adult hippocampus.

Hippocampal adult neurogenesis

Hippocampal structure and connectivity

The hippocampus is a brain structure located in the medial temporal lobe of the brain. It belongs to the limbic system and plays a very important role in learning and memory. The hippocampus is composed of the dentate gyrus (DG) and the Cornus Ammonis (CA), divided in three parts: CA1, CA2 and CA3 (Figure 2). Neurons from the entorhinal cortex (EC) project to the dentate gyrus via the perforant path, called this way because the axons of the entorhinal cortex perforate the subiculum to project on the granule cells of the dentate gyrus. The granule cells project on the pyramidal cells of the CA3 via their mossy fibers, and the neurons of the CA3 region project on the granule cells of the CA1 via Schaffer collaterals. The CA1 sends axons back to the entorhinal cortex. This perforant path-DG-CA3-CA1 pathway is also called trisynaptic circuit. The entorhinal cortex also directly projects on the CA1 and the CA3, and neurons of the CA3 are recurrently connected (Figure 2).

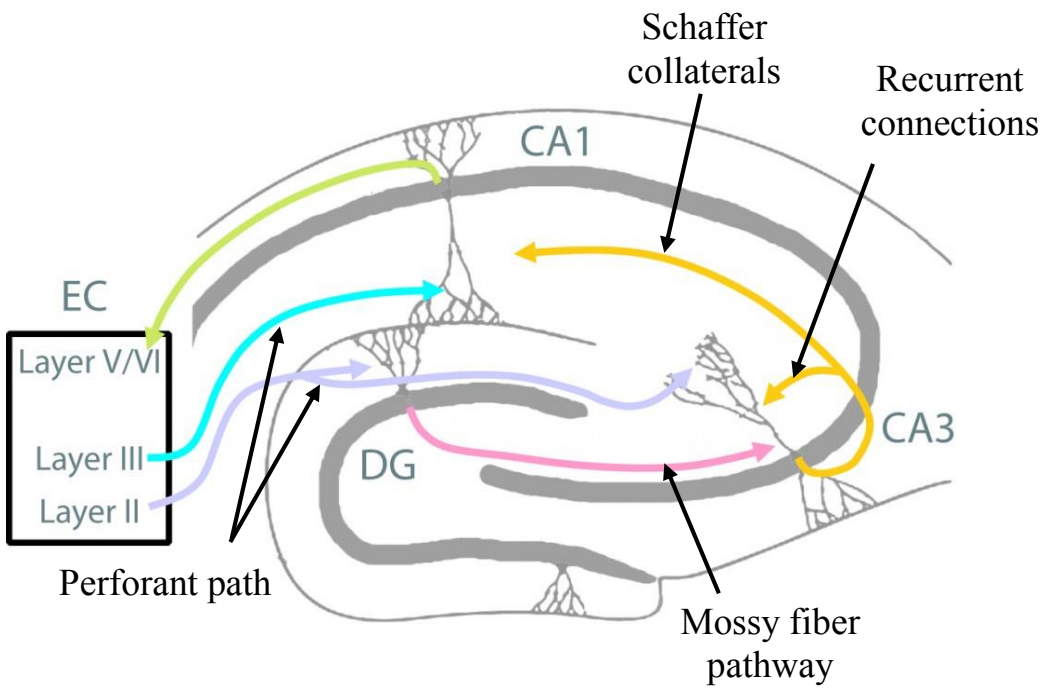


Figure 2: Hippocampal connectivity. The hippocampus is composed of the dentate gyrus (DG) and the Cornus Ammonis (CA), divided in three parts: CA1, CA2 and CA3. Neurons from the entorhinal cortex (EC) project to the DG via the performant path. The granule cells of the DG project to the pyramidal cells of the CA3 via the mossy fiber pathway, and the neurons of the CA3 project on the granule cells of the CA1 via Schaffer collaterals. Adapted from http://neuralcircuits.uwm.edu/neural-circuits-of-the-hippocampus/.

Location of hippocampal adult neurogenesis

Hippocampal adult neurogenesis was discovered in 1965 by Altman and Das 3, and its existence was confirmed in humans by Eriksson and colleagues in 1998 16. In physiological conditions, only one type of neuron is produced in the hippocampus: the granule cell of the dentate gyrus (Figure 3). The dentate gyrus is formed by three layers. The closest layer to the cortical surface is the molecular layer, which contains mainly dendrites and axons. Below it lays the granule cell layer, containing the cell bodies of granule cells. These cell bodies are densely packed, giving a V-shaped structure to this layer. Below the granule cell layer lays the hilus, formed by the axons of granule cells and interneurons. The subgranular zone (SGZ) is located between the hilus and the granule cell layer (GCL), and it contains the cells bodies of adult neural stem cells, which give rise to adult-born neurons (Figure 3).

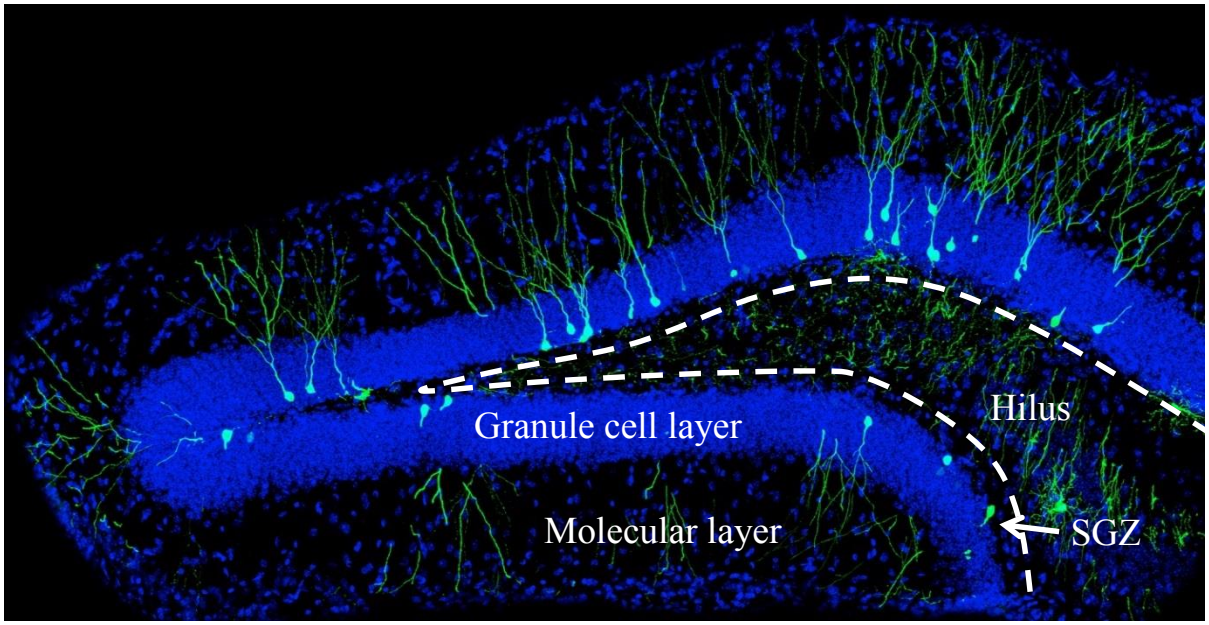


Figure 3: Structure of the mouse dentate gyrus. The mouse DG is formed by the molecular layer, which contains mainly dendrites and axons from the performant path axons of the entorhinal cortex, the granule cells layer, containing the cell bodies of granule cells, and the hilus, formed by the axons of the granule cells and interneurons. The subgranular zone (SGZ) contains the cell bodies of adult neural stem cells. The dashed white line represents the inferior limit of the SGZ. This limit is usually defined as being 3 cell bodies (i.e. about 40 µm) away from the limit of the granule cell layer.

Genesis, maturation and synaptic integration of hippocampal adult-born granule cells

Genesis and maturation of hippocampal adult-born neurons

In the mouse hippocampus, the formation and maturation of new neurons lasts for about 8 weeks. Newborn granule cells originate from the division of adult neural stem cells. Adult neural stem cells, also called type 1 cells, are a subpopulation of the radial glia of the SGZ and they express the same markers as astrocytes: the intermediate filament protein Glial fibrillary acidic protein (GFAP), Glutamate aspartate transporter (GLAST), glutamine synthetase and the calcium-binding protein S100-beta. They also express Brain lipid binding protein (BLBP), the intermediate filament protein Nestin, and Sox2, a transcription factor that is essential for maintaining self-renewal, that are commonly used as markers of neural stem cells (Figure 4). Their cell body is located in the SGZ (Figure 3) and they extend a radial process through the granule cell layer, which branches into the inner portion of the molecular layer. They self-renew by dividing symmetrically, but an adult neural stem cell can also divide asymetrically and give rise to an early progenitor cell and a new adult neural stem cell.

Early progenitor cells, also called type 2 cells, are divided in two groups: type 2a cells, that still express glial markers: GFAP, Nestin and Sox2, but lack the characteristic morphology of radial glia, and type 2b cells, that show the first indications of neuronal lineage choice and that express NeuroD1 and Prox1 (Figure 4). These neuronal precursors quickly proliferate, migrate and give rise to neuroblasts. It is important to note that this migration remains limited to the second third of the molecular layer.

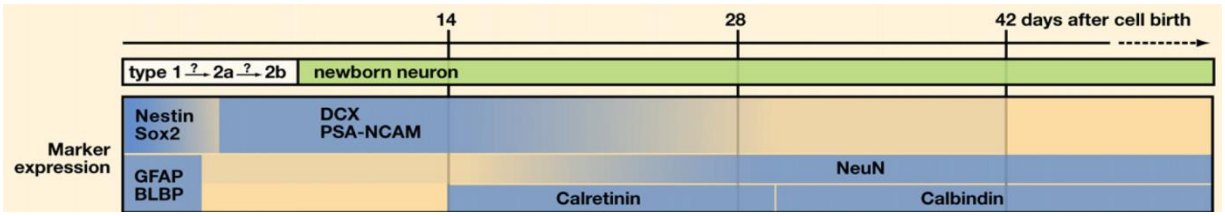
During this period of high proliferation, type 2b cells start expressing the microtubule-associated protein doublecortin (DCX) and Polysialylated-neural cell adhesion molecule (PSA-NCAM), which are markers of immature neurons, and stop expressing Nestin (Figure 4). This stage is called neuroblast, and corresponds to the type-3 cell.

Neuroblasts then start expressing NeuN, which is a neuronal marker, as well as the calcium-binding protein calretinin. During the second week after cell division, they start to extend their axonal processes to the hilus and their dendritic processes towards the molecular layer of the DG (Figure 4). At the end of the second week after birth, the first dendritic spines and mossy fiber terminals (MFT) of adult-born neurons are formed.

During the third week, newborn neurons continue to extend their axons and dendrites and to form spines in the molecular layer and MFTs in the CA3, and they stop expressing DCX and PSA-NCAM. At 4 weeks, they are fully integrated in the synaptic network, they stop expressing

the calcium-binding protein calretinin and start expressing the calcium-binding protein calbindin (Figure 4). Then, although neuronal maturation is complete, dendritic spines and axon terminals continue to mature until 8 weeks after birth. At the end of this period, adult-born neurons are undistinguishable from neurons born during the embryonic development, functionally and morphologically [18,19]. For more detailed information, please see the book chapter *Adult neurogenesis in the adult hippocampus*, from *Adult neurogenesis*, edited by F.H. gage, G. Kempermann and H. Song[20].

a



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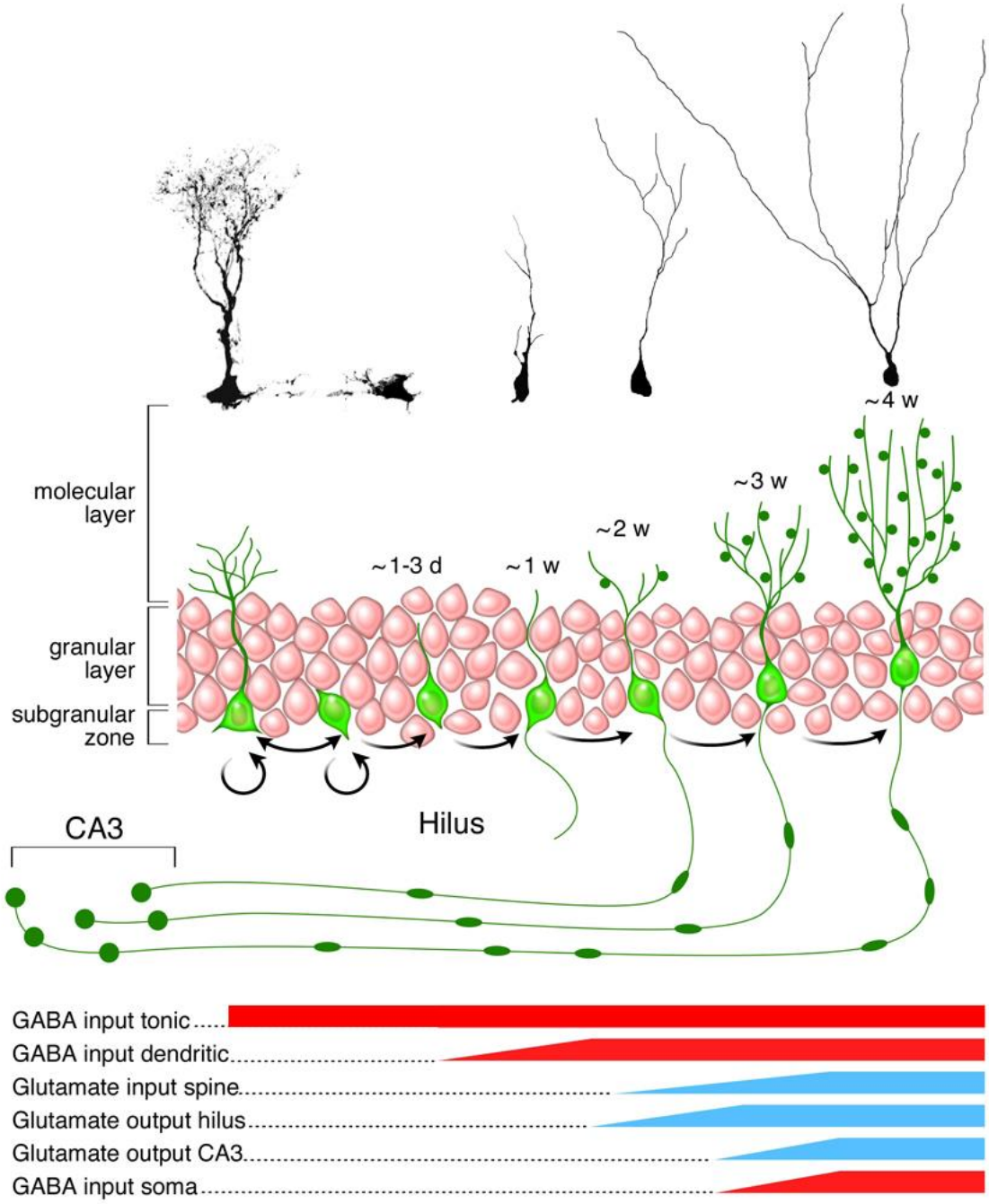


Figure 4: Time course of maturation of mouse hippocampal adult-born neurons. a, Marker expression of neuronal precursor cells and newborn neurons. Nestin, Sox2, GFAP and BLBP are expressed by adult neural stem cells. During the early progenitor stage (type-2a and type-2b cells), the cells stop expressing these markers and start expressing DCX and PSA-NCAM. From 14 days to one month after cell birth, neuroblasts express the calcium-binding protein calretinin. From one month after cell birth,

newborn neurons express the calcium-binding calbindin. NeuN is expressed by newborn neurons from the early progenitor stage, but is expressed at high levels only from one month after cell birth. b, Schematic representation and confocal micrographs of the different stages of morphological maturation, from stem cell to mature neuron. Full morphological maturation of newborn neurons is reached at 4 weeks after cell birth, although the synapses made by these neurons continue to mature until 8 weeks after cell birth. The bottom panel indicates the approximate timeline of the major input and output of adult-born neurons. The first input adult-born neurons get is GABAergic. Glutamatergic synapses only appear at the beginning of the third week of maturation, and glutamatergic input and output develop concomitantly. The final step of maturation of adult-born neurons is the formation of perisomatic GABAergic synapses at the end of the fourth week. Adapted from Zhao *et al.* 2008 and Toni *et al.* 2010 [21,22].

It is of note that in non-human primates, the time course of neuronal maturation in the adult hippocampus is longer: in adult macaque monkeys, granule cell maturation takes more than 6 months [23]. This lengthened time course for granule cell maturation may be relevant for preservation of neural plasticity over their longer lifespan. Also, given the phylogenetic proximity between humans and non-human primates, this work suggests that the period of maturation of hippocampal adult-born neurons may be longer in humans too.

During their maturation, adult-born neurons undergo a period of intense cell death: within the first four weeks of their development, 70% newborn granule cells die by apoptosis [24]. This suggests the existence of a mechanism of selection: neurons relevant for the function of the network may survive, while other neurons may die. Interestingly, the different steps of the generation, maturation and survival of adult-born neurons are regulated separately: an increase in proliferation does not necessarily lead to an increase in overall adult neurogenesis, and adult-born neuron survival can be increased without affecting proliferation. For example, exposure to an enriched environment promotes the survival of progenitor cells in C57BL6 mice without affecting their proliferation [25]. Conversely, antidepressants increase hippocampal neural progenitor proliferation without affecting their survival in adult rats [26].

Tools to study the generation and maturation of adult-born hippocampal neurons

Different techniques are used to study adult-born hippocampal neurons and their maturation. [^{3}H]thymidine is incorporated into the DNA of cells during DNA synthesis, allowing for the relatively specific labeling of cells in S phase of the cell cycle. In the original publications on

adult neurogenesis, tritiated thymidine ([^{3}H]thymidine) was used to investigate the time of origin of hippocampal neurons. However, inherent limitations of the technique, such as the radioactivity of this substance and the time-consuming nature of autoradiography, coupled with the inability to sample more than the upper few micrometers of a tissue section, led to the development of other approaches.

Nowadays, the thymidine analog bromodeoxyuridine (BrdU) is used for labeling cells in division. Similarly to tritiated thymidine, BrdU incorporates into the DNA of cells engaged in DNA synthesis. Immunohistochemistry using a monoclonal antibody directed against this molecule gives this method several inherent advantages over [^{3}H]thymidine autoradiography, including the ability to amplify the signal and detect labeled cells throughout thick tissue sections. In addition, fluorescently labeled secondary antibodies allow colocalization of BrdU with other cell-type markers, such as NeuN, to identify adult-born neurons. This technique was pivotal in confirming the existence of neurogenesis in the human adult hippocampus [16]. Animals usually undergo several injections of BrdU during a short period of time, to label a consequent number of cells, and progenitor cell proliferation can be evaluated by sacrifying the animals up to one day later, whereas granule cell survival is assessed by sacrifying the animals at later timepoints. Newborn neurons are detected by co-immunohistochemistry against BrdU and neuronal markers such as DCX, that labels immature neurons from 1 to 3 weeks after cell division, and NeuN, that labels mature neurons. However, this method does not give access to the morphology of newborn neurons, and does not allow the selective identification of neurons at specific developmental stages.

To achieve this goal, transgenic reporter mouse lines have been used. Nestin expression marks stem and progenitor cells in the developing and adult central and peripheral nervous system. Regulatory elements of the nestin gene have been used by several groups to generate reporter transgenic lines that express GFP or other fluorescent proteins in neural stem and progenitor cells of the adult nervous system. These mouse lines are used to study the very first steps of adult neurogenesis, i.e. stem cell division and neuronal progenitor proliferation. Similarly, the promoter of the DCX gene has been used to generate reporter transgenic lines expressing fluorescent proteins in neural progenitor cells and immature neurons until three weeks of development. DCX is expressed in immature neurons from one to three weeks of development, thus these transgenic lines are useful to study intermediate steps of adult-born neuronal maturation. Transgenic mouse lines have several advantages: they are non-invasive, and they allow full morphological analysis of the cells. Furthermore, reporter mice can easily be crossed

with other genetically modified mice to examine the role of specific genes or mutations on adult neurogenesis. However, specificity of marker expression in newborn cells must be confirmed with other approaches, because some developmentally regulated proteins might be re-expressed in mature neurons that undergo functional or structural changes. Also, since transgenic labeling identifies cells based on their ability to express a stage-specific marker, transgenic reporter lines provide a snapshot of a population of cells at a particular time window of their development, but do not allow individual cells to be tracked over time.

In contrast, retroviral labeling allows tracking newborn cells over time. Retroviruses are characterized by their ability to generate double-stranded DNA (dsRNA) from their RNA genome through reverse transcription. Upon entry into host cells, the retroviral RNA genome is transcribed into a dsDNA in the cytoplasm of the host cell. The newly synthesized dsDNA enters the nucleus, where it integrates into the chromosomal DNA of the host cell. Oncoretroviruses, such as Moloney murine leukemia virus (MoMuLV), enter the nucleus at the onset of mitosis, when the nuclear envelope breaks down, and as a consequence only transduce dividing cells. These viruses have been pseudotyped to infect dividing neuronal progenitor cells only, and then used to label these progenitors during neurogenesis. Because retrovirus-mediated cell labeling results in permanent labeling of dividing cells and their progeny, and because neural progenitors give rise to adult-born neurons, this technique has enabled the examination of the morphological development and the functional integration of newborn neurons *in vivo*.

By injecting a retrovirus carrying the expression cassette of a fluorescent protein reporter gene in the dentate gyrus of adult mice, van Praag and colleagues were the first to show that one-month-old adult-born dentate granule cells become functional and integrate into the hippocampal network [27]. Next, using the same methodology and sacrifying the mice at different timepoints of newborn neuron maturation, Zhao and colleagues could follow the morphological and functional development of adult-born neurons [28].

Also, retroviruses can be modified to alter the expression of a gene of interest, so that the function of the gene can be studied in the context of progenitor proliferation, differentiation, and neuronal maturation. For example, different forms of a protein of interest, including wild-type, constitutively active, and dominant-negative forms can be overexpressed. Genes of interest can also be knocked down using RNAi. This strategy allows the cell-autonomous analysis of individual neurons because only small populations of cells are genetically modified and their surrounding environment is intact.

However, retrovirus-mediated cell labeling in the adult rodent brain requires stereotaxic surgery to deliver the virus to the area of interest. This approach is thus invasive, and the surgery itself can induce local inflammatory reactions, that may bias the results. Also, retroviral labeling labels a little number of cells, which may be a problem for detecting small size effects, or for studying larger-scale effects of the genetic modification of adult-born neurons, such as effects on animal behaviour.

New transgenic approaches that exploit inducible and conditional expression systems, such as tamoxifen-dependent recombination (e.g, using Cre-ER, a fusion of Cre recombinase and the hormone-binding domain of the oestrogen receptor) or tetracyclin-dependent gene expression or recombination (e.g., using Tet-based activators) combine the advantages of both genetic and retroviral labeling, and provide more powerful methods to label cells and manipulate adult neurogenesis. For an exhaustive review of the topic, please see the book chapters *Detection and phenotypic characterization of adult neurogenesis*, *Evolving methods for the labeling and mutation of postnatal neuronal precursor cells: a critical review*, *The use of reporter mouse lines to study adult neurogenesis*, and *Retrovirus-mediated cell labeling* from *Adult neurogenesis,* edited by F.H. gage, G. Kempermann and H. Song[20].

Synaptogenesis and synaptic activity of hippocampal adult-born neurons

Adult-born neurons become functional: using a retroviral vector carrying a GFP expression cassette to label adult-born hippocampal neurons, van Praag and colleagues showed that they display passive membrane properties, action potentials and functional synaptic inputs [27] (Figure 5).

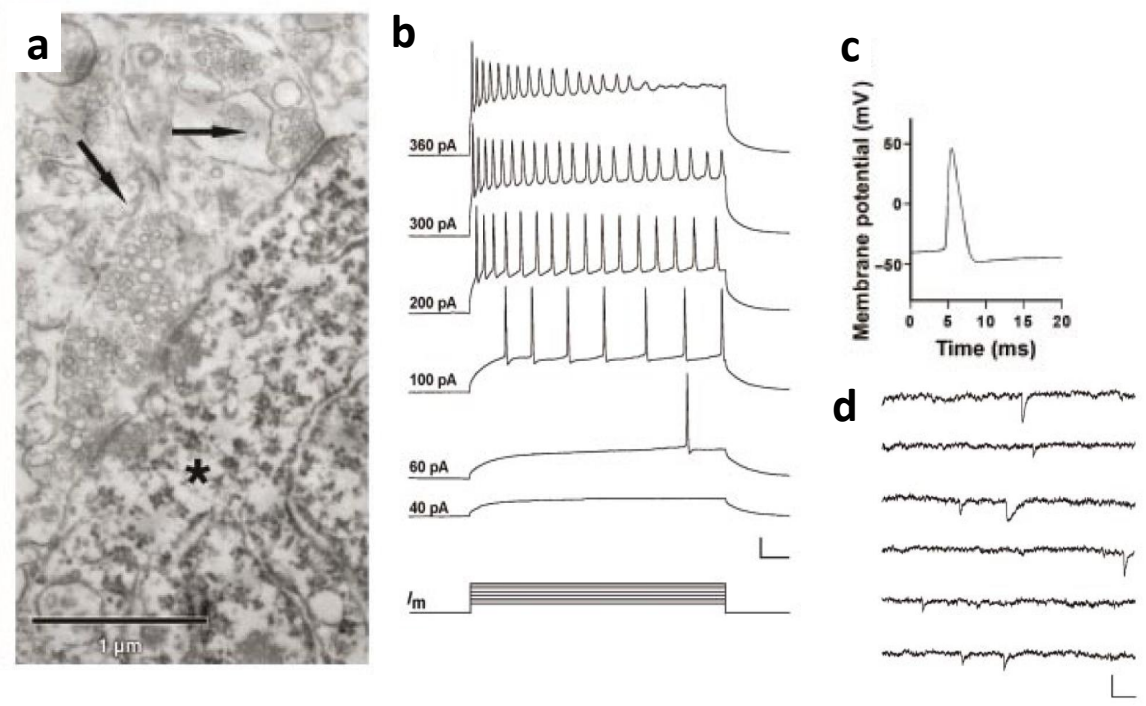


Figure 5: Newly generated neurons receive synaptic inputs and display neuronal electrophysiological properties [25]. a, Electron micrograph of synaptic terminals (arrows) on the soma of an adult-born neuron (asterisk) in the granule cell layer. b, Membrane potential of an adult-born neuron in response to depolarizing currents recorded under current clamp at the resting potential. Numbers on the left indicate stimulus size. Scale: 25 mV, 50 ms. c, Action potential recorded in an adult-born neuron. d, Spontaneous postsynaptic currents recorded under voltage clamp.

How are the synapses of adult-born neurons formed?

While still dividing, early progenitors show tonic activation by immature GABAergic inputs from parvalbumin interneurons (Figure 4). During the first days of maturation, even if they lack synaptic inputs, newborn neurons express $GABA_A$ and glutamate (both α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid i.e. AMPA and N-methyl-D-aspartate i.e. NMDA) receptors, as well as voltage-dependent Na^+ and K^+ channels at low levels. As a consequence, depolarizing steps elicit immature action potentials (single spikes with small amplitude and long duration) in current clamp recordings. Recently, Song and colleagues showed that early progenitors quickly form immature synapses with parvalbuminergic interneurons. Surprisingly, the role of these synapses seems related to progenitor survival, and not to synaptic transmission: activation of parvalbuminergic interneurons promotes the

survival of early hippocampal progenitors, whereas suppression of parvalbuminergic neuron activity decreases their survival [29].

Through the second week of maturation, GABAergic terminals make the first functional synapses onto the dendrites of new neurons (Figure 4). Presynaptic GABA release now elicits depolarizing post-synaptic currents with slow kinetics [30-32]. One important population of GABAergic neurons responsible for the initial contacts with slow kinetics are the neurogliaform interneurons, whose connectivity with adult-born neurons is initially sparse, and increases several fold as neurons mature [33].

Until the second week of adult-born neuron development, GABA acts as an excitatory transmitter. GABA triggers conformational changes in $GABA_A$ receptors, which function as ligand-gated chloride (Cl^-) channels, to facilitate the passive inflow or outflow of Cl^- ions. The driving force of Cl^- (ICl^-) is proportional to the difference between the membrane potential (Vm) and the reversal potential of Cl^- (ECl^-), $Vm-ECl^-$. When $Vm>ECl^-$, $Vm-ECl^->0$ so $ICl^- > 0$ and this leads to an influx of Cl^- in the neuron. When $Vm<ECl^-$, $Vm-ECl^-<0$ so $ICl^-<0$ and this leads to an efflux of Cl^- from the neuron. The regulation of intracellular Cl^- concentrations in neurons depends primarily on the balance between the activity of the $Na^+/K^+/Cl^-$ cotransporter NKCC1, that drives Cl^- influx, and the Cl^-/K^+ cotransporter KCC2, that drives Cl^- extrusion. Until two weeks of development, NKCC1 levels of expression in immature neurons are high and KCC2 levels of expression are low, leading to a high intracellular Cl^- concentration, and, in turn, a high ECl^-. As a consequence, in neurons before 2 weeks of maturation, $Vm<ECl^-$, so $ICl^-<0$ and $GABA_A$ receptor activation by GABA triggers Cl^- efflux, which leads to membrane depolarization. As neurons mature, KCC2 levels progressively increase, and ECl^- value becomes inferior to Vm value. This produces a GABA shift: at the beginning of the third week of maturation, $ICl^->0$, and $GABA_A$ receptor activation now induces Cl^- influx and membrane hyperpolarization. GABA becomes hyperpolarizing, acting as an inhibitory neurotransmitter [34-36].

The first glutamatergic synapses appear when the neurons enter the third week of maturation, at the same time as they form their first mossy fiber terminals [19,28] (Figure 4). The mechanism of onset of glutamatergic synaptogenesis has been discovered recently: Chancey and collaborators showed that immature granule cells bear NMDA receptors only-containing synapses. These synapses are silent, and can be converted into NMDA/AMPA active synapses after coincident GABA-mediated depolarization and NMDA receptor activation by presynaptic

glutamate release [37]. This unsilencing leads to the production of functional glutamatergic synapses.

Glutamatergic synaptogenesis onto developing neurons takes several weeks. As granule cells grow, dendrites grow and branch, and spine density increases [19,28,38] (Figure 4). This morphological development is accompanied by an increase in the amplitude of postsynaptic responses that can be visualized upon activation of the performant pathway in acute slices. The maximal size of excitatory postsynaptic responses is reached after six weeks of maturation [18,39,40]. The final step of maturation of adult-born neurons is the formation of perisomatic GABAergic synapses at the end of the fourth week [40] (Figure 4). For more detailed information, please see Toni and Sultan 2011[21] and Toni and Schinder[41], in press.

Thus, during their development, adult-born neurons make excitatory synapses with perforant path afferences in the molecular layer on their dendritic spines, and their axon terminals synapse with pyramidal neurons of the CA3. This involves plasticity and remodeling of the pre-existing afferent and efferent neurons. This raises the following question: does the adult brain generate new pre- and post-synaptic partners to accommodate the new neurons, or do new neurons connect to pre-existing partners?

The work of Nicolas Toni and colleagues supports the second hypothesis [19,21,42]. Indeed, three-dimensional reconstructions of mature dendritic spines based on serial section electron micrographs showed that they formed multiple synapse boutons, i.e. they contacted axon terminals already synapsing with one or several other neurons. At 4 weeks after cell division, two thirds of the dendritic spines of adult-born neurons contacted multiple-synapse boutons (MSB), whereas one third contacted a bouton devoid of other synapse. Also, the proportion of dendritic spines contacting a MSB decreased with adult-born neuron maturation, suggesting a transformation of MSBs into single-synapse boutons (SSB) over time.

Interestingly, the development of MFTs from adult-born neurons follows a similar mechanism [42]. The immature axon terminals contact the dendritic shafts of CA3 pyramidal cells. When they mature, the MFTs contact either individual small thorny excrescences protruding from these dendrites, or share mature thorny excrescences with other granule neurons. It is only after two months of maturation that MFTs contact individual and mature thorny excrescences. This suggests that both the synaptic input and output of adult-born neurons have a time window during which synaptic partners are shared with pre-existing neurons, and that they compete with

those of pre-existing neurons and finally replace them at synapses (Figure 6). Interestingly, most of the adult-born hippocampal neurons die by apoptosis within the first four weeks of their maturation [24], and this cell death correlates with glutamatergic synaptogenesis. Together, these findings suggest that adult-born neurons may compete with other neurons for survival.

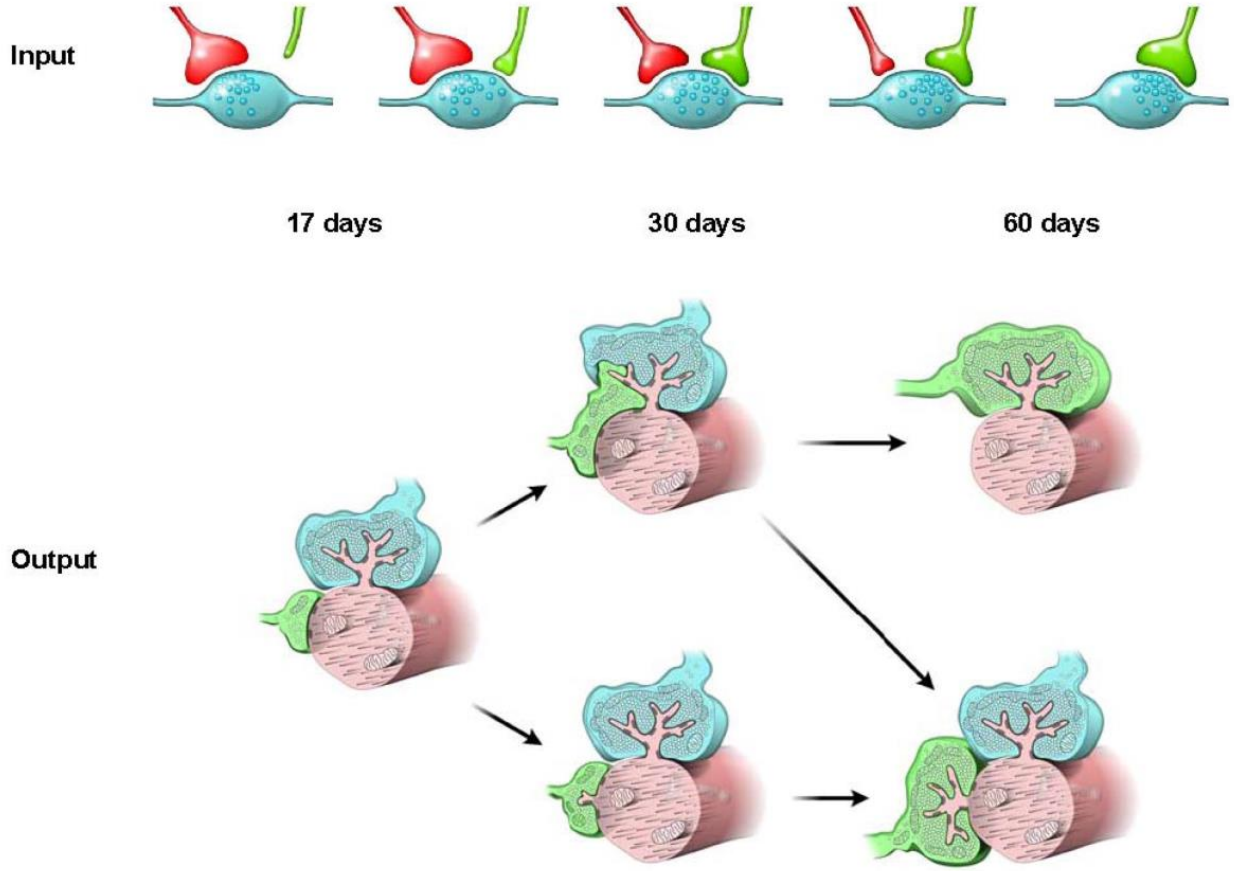


Figure 6: Suggested sequence of events involved in the synaptic integration of adult-born neurons into the glutamatergic network. Upper panel (input): A filopodia of an adult-born neuron (green) is attracted by a pre-existing synapse between an axonal bouton (blue) and another neuron (red). When the filopodia stabilizes and matures into a dendritic spine, a MSB is formed. Progressively, the spine from the adult-born neuron increases in size and the spine from the other neuron decreases in size until it retracts, transforming the MSB in a SSB. Lower panel (output): Upon reaching the CA3 area, MFTs contact the dendrites of pyramidal cells. At about one month, MFTs contact thorny excrescences, some of which are shared with pre-existing neurons and it is only after 2 months that individual contacts are made between pre- and post-synaptic partners. From Toni *et al.* 2011 [21]

Thus, hippocampal adult-born neurons become functional, and follow a specific time course of maturation and synaptogenesis. As the energetic cost of the production of new cells by an

organism is high, the fact that adult hippocampal neurogenesis is quantitatively important and has been evolutionary conserved suggests that it plays important physiological functions. Do hippocampal adult-born neurons have specific properties that give them a specific physiological role?

Hippocampal adult neurogenesis is involved in learning and memory

Long-term potentiation (LTP) is a long-lasting enhancement in signal transmission between two neurons that results from stimulating them synchronously. Compared to mature neurons, immature adult-born neurons display enhanced plasticity and a lower threshold for LTP induction: Schmidt-Hieber and colleagues showed that long-term potentiation could be induced more easily in young neurons than in mature neurons under identical conditions (Figure 7) [43]. Indeed, when the same stimulation paradigm was applied to both cells, only immature neurons showed a significant increase in excitatory post-synaptic potential amplitude.

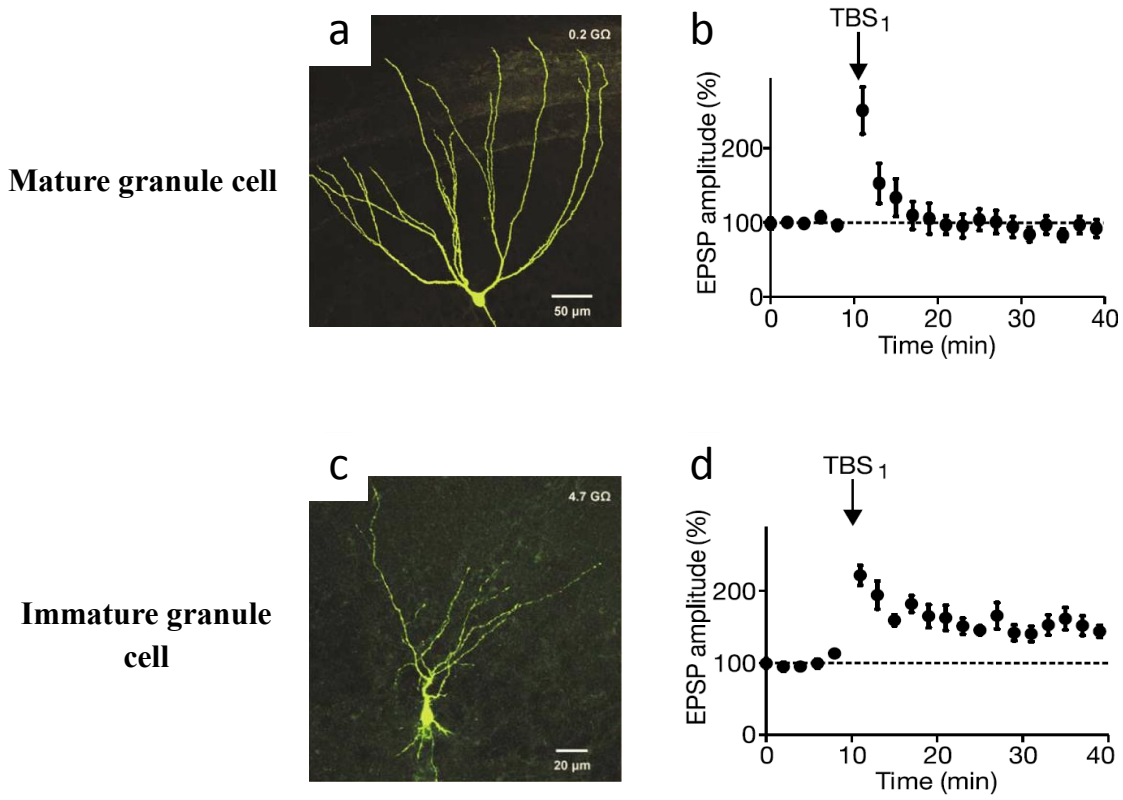


Figure 7: Young granule cells have a lower threshold for LTP induction than mature granule cells. a, c, Biocytin-filled mature (a) and young (c) granule cell. b, d, average excitatory post-synaptic potential

amplitude for mature (b) and young (c) granule cells. The same paradigm of LTP induction (TBS1) was applied to both neurons. Adapted from Schmidt-Hieber *et al.* 2004 [43]

However, adult-born neurons only show increased synaptic plasticity during a specific time window, i.e. between 3 and 7 weeks after neuronal birth[44]. Then, their level of plasticity becomes similar to that of mature granule cells. Different synaptic and network mechanisms seem to contribute to the enhancement in synaptic plasticity observed in immature neurons. The key mechanism is the switch in the NMDA receptor composition occurring during neuronal maturation. Immature neurons express NMDA receptor 2 subunit (NR2B)-containing NMDA receptors, which display high affinity for CaM kinase II (CaMKII) and are responsible for the lower threshold of LTP induction in adult-born neurons. As neurons mature, they switch towards NR2A-containing NMDA receptors that present lower affinity for CaMKII and, as a consequence, decreased levels of LTP expression. Interestingly, the enhanced plasticity observed for excitatory inputs has been recently demonstrated for the outputs of newborn neurons, at the level of mossy fibers[45].

This enhanced plasticity confers a particular role to adult-born neurons in learning and memory. In fact, adult neurogenesis seems to be particularly important for performance in complex learning tasks. As we will see with several examples below, the experimental reduction of adult neurogenesis in rodents results in learning and memory impairments, whereas increasing adult neurogenesis improves learning and memory.

Volontary wheel running and environmental enrichment robustly increase hippocampal adult neurogenesis[46,47]. Using a combination of toys, food supplement, running-wheel and a wider cage, Kempermann and colleagues showed that the survival of newly-born granule cells in the dentate gyrus increased in adult C57BL6/J mice housed in an enriched environment versus in standard laboratory cages. Similarly, van Praag and colleagues showed that voluntary wheel running increases proliferation and overall adult neurogenesis in mice: running doubled the number of surviving newborn hippocampal neurons in adult mice, in amounts similar to enriched environment.

Incidentally, environmental enrichment and running improve learning and memory. van Praag and colleagues tested LTP and spatial learning in the Morris water maze in groups of mice housed with a running wheel or under standard conditions. The Morris water navigation task consists in training the mice to find a hidden platform that allows them to escape from the water.

A shortening of the time to find the platform over trial indicates that the mice learnt the position of the platform. Following training, spatial memory is evaluated with a probe test, in which the platform is removed from the pool. The time the mouse spends searching the platform in the quadrant where it was located previously (target quadrant) is measured and compared to the time the mouse spends in the other quadrants. More than 25% of the total time spent in the target quadrant is indicative of memory retention. They found that voluntary wheel running enhanced dentate gyrus LTP, and improved learning and memory performances, as the time spent searching the platform during training decreased faster during training for running than non-running mice [48].

Similarly, environmental enrichment enhances memory function in the water maze and other various learning tasks [49-51]. Thus, voluntary running and environmental enrichment increase adult neurogenesis as well as learning and memory performances, suggesting a relationship between adult neurogenesis and cognition.

After ablating adult neurogenesis by irradiation in mice, Clelland and colleagues found impairments in spatial discrimination in two behavioral assays: a spatial navigation radial arm maze task and a spatial, but non-navigable task in the mouse touch screen [52]. The navigation radial arm maze task they used comprises 3 phases (Figure 8a). During the habituation phase, mice are placed in a radial maze in which all arms are unblocked and all wells at the end of the arms contain one food pellet each, so that they learn that the end of each arm contains a single food pellet. During the sample phase, mice are placed in the maze where all arms except the start arm and the sample arm are blocked. The mouse is allowed to visit the sample arm and to retrieve a food pellet reward. During the choice phase, arms in the start and sample (unrewarded) locations are open, as well as an arm in an additional correct location (rewarded). As the mouse previously learnt that each arm only contains a single food pellet, it is expected, if it makes the difference between the sample and the additional location, to go to the additional arm. Additional arms varied in distance from the sample arm by a spatial separation of 2, 3, or 4 arms (Figure 8b). Mice with ablated hippocampal neurogenesis were selectively impaired at low separations (spatial separation of 2 arms), but not at high separations (separation of 3 or 4 arms) (Figure 8d).

In the mouse touch screen task, the mice are placed in front of a screen containing 5 squares, and are required to touch one of the two illuminated squares with their nose (the one on the left) to receive a reward, until a criterion (7 correct touches out of 8 consecutive touches) is reached

(Figure 8c). Mice were tested on either the low separation (illuminated squares separated by one dark square) or the high separation (illuminated squares separated by 3 dark squares). Mice with ablated neurogenesis exhibited impaired performance at low but not high separations during the acquisition of this task, consistent with a pattern separation deficit observed in the first experiment (Figure 8e).

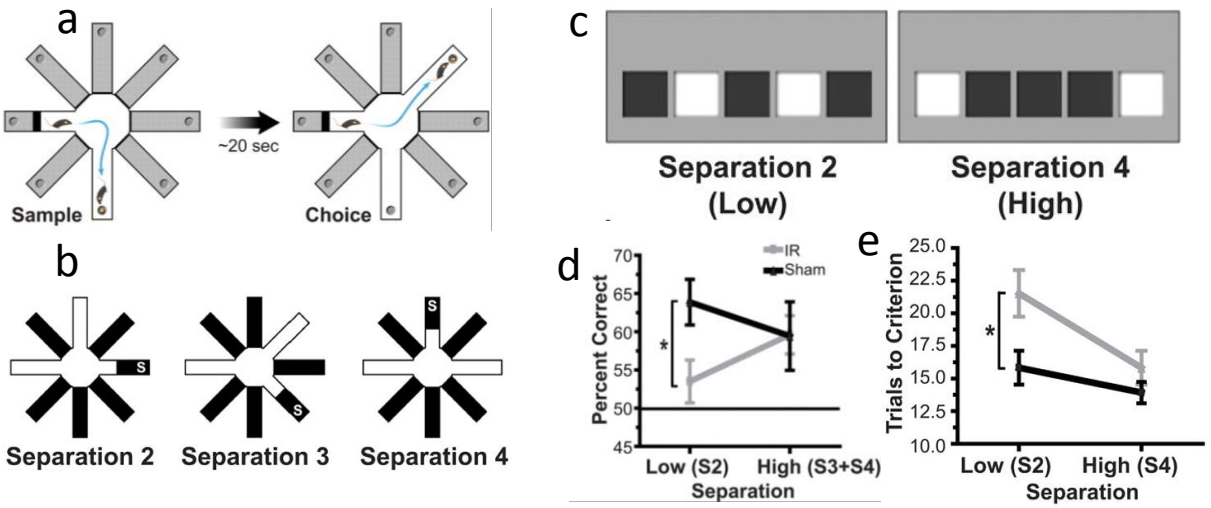


Figure 8: Mice with ablated neurogenesis show impaired pattern separation. a, Each trial in the radial maze consists in a sample phase and a choice phase. During the sample phase, the mouse retrieves a food pellet in the only accessible arm. During the choice phase, the mouse can go either the sample or to the newly unblocked arm, and is expected to go directly to the new arm. b, The difficulty of the pattern separation task in the radial maze is increased by increasing the distance between the sample and the new arm. c, In the mouse touch screen task, the mice has to touch the correct square with its nose, e.g., the left illuminated square of a screen containing two illuminated squares and 3 unlit squares. The experiment stops when a criterion of 7 of 8 consecutive correct touches is reached. Mice are tested on either the low separation (Separation 2) or the high separation pattern (Separation 4). Adapted from Clelland *et al.* 2009 [52]

Therefore, mice with ablated hippocampal adult neurogenesis were impaired when stimuli were presented with little spatial separation, but not when stimuli were more widely separated in space and therefore easier to distinguish. This shows that adult-born neurons are involved in pattern separation function in the dentate gyrus of adult mice.

The most compelling evidence for a role of adult neurogenesis in learning and memory was obtained recently by optogenetics. Indeed, combined optogenetics and viral approaches allow selective activation or inhibition of a population of adult-born neurons of the same age during a short period of time. Alonso and colleagues optogenetically activated adult-born neurons of the olfactory bulb and tested the mice with a task of odor recognition [53] (Figure 9). This task consisted to partially water-deprive mice, and to expose them to two different odors. When the mice were exposed to odor 1, they received water supply if they licked the water delivery tube in front of them. In contrary, odor 2 was not associated with water delivery. Therefore, if the mouse could discriminate odor 1 and odor 2, it was expected to lick the water tube only when it smelled odor 1. Mice were tested with different pairs of odors, some easy to discriminate (two different molecules) and some harder to discriminate (two enantiomers of the same molecule). Mice with optogenetical activation of adult-born neurons simultaneous to odor delivery learnt faster and remembered better than control mice, but only in the context of a difficult pair of odors. Therefore, learning and memory were improved by the optogenetical activation of adult-born neurons, but only in the context of a difficult task of odor recognition.

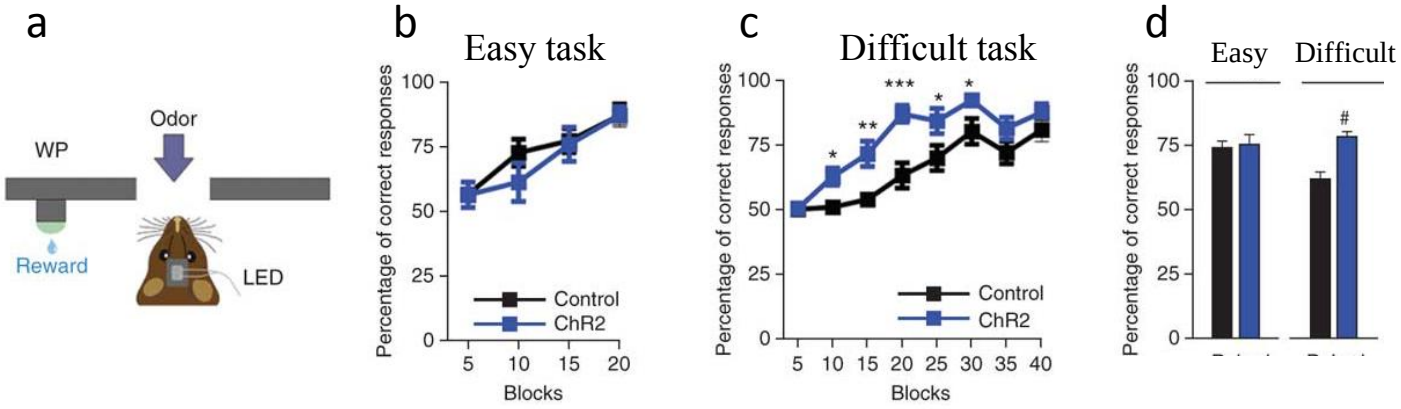


Figure 9: Optogenetical activation of adult-born neurons in the olfactory bulb accelerates learning and improves memory. a, Partially water-deprived mice were trained to lick the water delivery tube only when smelling odor 1. Odor 2 was not associated with water delivery. The percentage of correct responses (lickings of the water tube when odor 1 was presented and absence of licking when odor 2 was presented) was recorded. Stimulation of adult-born neurons simultaneous to odor delivery did not change the learning rate of an easy odor discrimination task (b), whereas a difficult task was learnt quicker (c). d, To assess olfactory memory, mice were tested 50 days after the odor discrimination

task. Adult-born neuron stimulation simultaneous to odor delivery improved memory, but only in the context of a difficult task. Adapted from Alonso *et al.* 2012 [53]

In the hippocampus, Gu and colleagues used optogenetics to reversibly silence groups of different aged adult-born hippocampal neurons during behavioral tasks[45]. They generated a retrovirus carrying the expression cassette of an inhibitory optogene, Archaerhodopsin-3. This gene encodes a light-driven outward proton pump. The activation of this pump by light produces hyperpolarizing currents and leads to effective and reversible neuron silencing[54]. This retrovirus selectively infects neuronal precursor cells, which allows the selective expression of the gene in a subpopulation of adult-born neurons of the same age.

The authors injected a retrovirus carrying the Arch-Green fluorescent protein (Arch-GFP) expression cassette in the dentate gyrus of adult mice to express Arch in hippocampal adult-born neurons. With this approach, they could reversibly silence hippocampal adult-born neurons at different timepoints of their maturation. Then, they tested the mice with two different behavioral assays: contextual fear conditioning and spatial learning in Morris water maze.

Silencing adult-born neurons during the time window during which they display increased synaptic plasticity, i.e. 4 weeks after cell division (4wpi), disrupted hippocampal memory retrieval in the two tests (Figure 10). Mice with inactivated adult-born neurons froze less than control mice in the context associated with the electrical shock (Figure 10a), and they spent an equal amount of time in the 4 quadrants of Morris water maze during the probe test (Figure 10b). On the other hand, silencing the neurons before (2 weeks i.e. 2 wpi) or after this time window (8 weeks i.e. 8 wpi) had no effect on memory retrieval (Figure 10c-d). This suggests that adult-born neurons play a specific role in learning and memory during the time window during which they display increased synaptic plasticity.

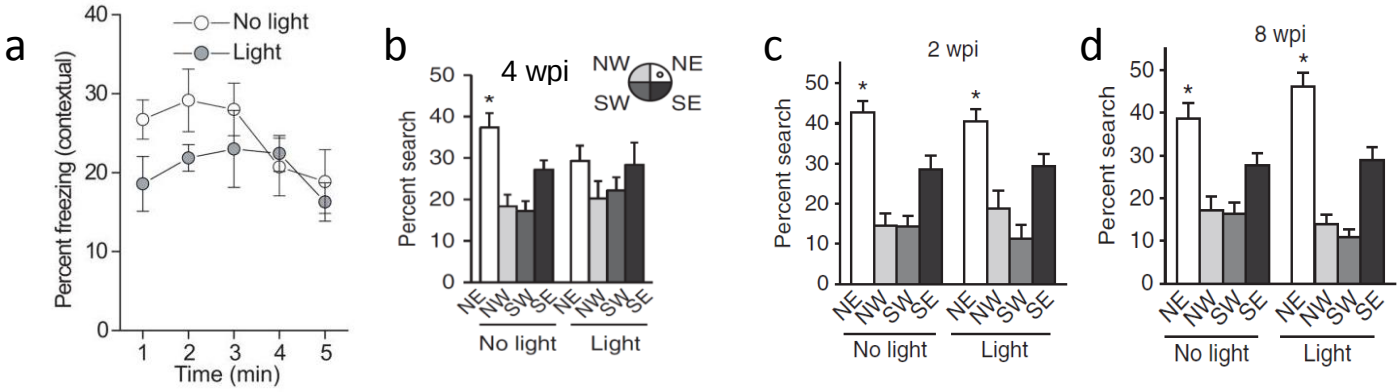


Figure 10: Temporary silencing of 4 week-old neurons impairs memory retrieval. a, Temporary silencing of 4 week-old adult-born neurons impairs the expression of a fear conditioning memory: silencing of adult-born granule cells at 4 weeks post-injection (4 wpi) reduced freezing to the context compared with controls (No light). b, Optical inactivation of 4-week-old adult-born neurons (4 wpi) impairs spatial memory. During the probe test, control mice spent significantly more time searching in the target quadrant (NE) compared with the other quadrants, whereas the difference was not significant for mice with inactivated adult-born neurons. Behavioral roles of adult-born neurons are sensitive to their age: silencing adult-born neurons at 2 weeks (2 wpi) (c) or 8 weeks (8 wpi) (d) after cell division did not affect memory retrieval. Adapted from Gu *et al.* 2012 [45]

Thus, adult-born neurons become functional and play a role in crucial brain functions, such as learning and memory. New granule cells preferentially make synapses with partners already synapsing with other neurons. Consequently, adult neurogenesis may be a mechanism of brain repair in the context of disease, as newborn cells may then replace dying pre-existing granule cells. Could adult neurogenesis be a mechanism of brain repair, or could neurological diseases influence adult neurogenesis, which would then participate to the pathogenesis?

Adult neurogenesis and pathologies

Alteration of adult neurogenesis in neurodegenerative diseases

Huntington's disease (HD) is a neurodegenerative inherited disorder with progressive symptoms, including involuntary movements, cognitive deficits and various psychiatric disturbances. The most striking feature of HD is the progressive degeneration of projection neurons, leading to atrophy of the striatum, which is adjacent to the SVZ. *Post-mortem* analyses of brains from HD patients showed an increased SVZ thickness due to increased cell proliferation. This increased cell proliferation was mostly due to an increase in the proliferation of neural stem cells in the SVZ[55,56]. This alteration of neurogenesis was precisely described in

a transgenic mouse model of HD, R6/2 mouse, which carries human HD mutations[57]. The ability of neural stem cells dissociated from the SVZ of this mouse to self-renew increased with the progression of the disease. In addition, a subpopulation of the neuroblasts from the SVZ migrated to the striatum, whereas the migration of neuroblasts toward the olfactory bulb was significantly reduced, suggesting that neuroblast migration was redirected from the olfactory bulb to the striatum. Unlike in the SVZ, cell proliferation and neurogenesis are decreased in the hippocampus of R6/2 mice[58,59]. The relationship between decreased hippocampal neurogenesis and the pathogenesis of HD has not been defined. However, enrichment of the mouse environment, known to increase hippocampal neurogenesis, delays the progression of the symptoms of the disease in the model mice [60].

Alzheimer's disease (AD) is one of the most common neurodegenerative diseases, and is characterized by the progressive alteration of cognitive functions and severe neurodegeneration. AD is characterized by the accumulation of β-amyloid peptide (Aβ) in senile plaques, and by neurofibrillary tangles, containing the hyperphosphorylated microtubule-associated protein tau. Aβ is derived from the amyloid precursor protein (APP). Animal models of AD produce conflicting data regarding adult neurogenesis. The majority of studies performed on transgenic animals expressing the mutant APP show a decrease in adult neurogenesis in the DG or both the DG and the SVZ [61]. The analysis of *post-mortem* brain tissues from AD patients showed a decrease in neuronal progenitor cells in the SVZ [62] but an increase in progenitor cells in the DG[63]. These conflicting results could be explained by the fact that animal models of AD do not perfectly reflect the pathology: the animals usually carry a single mutation involved in familial AD, and they do not reproduce all the symptoms of the disease [64]. Also, data from *post-mortem* human tissue are difficult to interpret because *post-mortem* material reflects the late stages of the disease. Importantly, drugs used to treat AD increase adult neurogenesis in animal models [65]. Overall, the complexity of AD pathogenesis prevents a clear understanding of the role and the direction of changes in adult neurogenesis, but it remains possible that a decrease in neurogenesis contributes to AD pathogenesis. For more detailed information, please see Kaneko and Sawamoto 2009[66].

Alteration of adult neurogenesis in neuropsychiatric disorders

Depression is the most common mental illness in the world and is characterized by pervasive and persistent low mood accompanied by low self-esteem and by a loss of interest or pleasure in

normally enjoyable activities. Exposure to long-term psychosocial stress and repeated inescapable stress leads to depressive behaviors in animals and suppresses the production and/or survival of adult-born neurons in the DG[67,68]. Interestingly, the chronic administration of antidepressants such as tricyclic antidepressants and serotonin-selective reuptake inhibitors (SSRI) and mood stabilizers increases hippocampal neurogenesis[26,69]. Also, when hippocampal neurogenesis is disrupted by irradiation, SSRI do not attenuate depressive behavior anymore, indicating that neurogenesis is necessary for the mechanism of action of antidepressants[70].

Schizophrenia is a mental disorder affecting about 1% of the general population, and is characterized by a breakdown in thinking, hallucinations, delusions, lack of motivation and poor emotional responses. *Post-mortem* studies of the human brain reported a more than 60% reduction in the number of proliferating cells expressing ki67, a cell-cycle marker, in the hippocampal SGZ of patients with schizophrenia[71]. Moreover, a schizophrenia susceptibility gene, DISC1 (Disrupted in Schizophrenia 1) plays a role in the regulation of the maturation and the integration of newly generated granule cells[72]. Furthermore, disruption of hippocampal neurogenesis by irradiation in mice leads to the behavioral abnormalities seen in animal models of schizophrenia [73], and there is a higher prevalence of schizophrenia among people accidentally exposed to radiation [74,75]. This suggests a link between hippocampal neurogenesis and schizophrenia, although the involvement of hippocampal neurogenesis in the neuropathophysiology of schizophrenia has not been established yet. For an exhaustive review of the topic, please see Kaneko and Sawamoto 2009[66].

Thus, adult neurogenesis correlates with learning and memory, and is altered in a number of pathologies. It is therefore likely that increasing adult neurogenesis might be an interesting therapeutic approach to treat some of these diseases or to restore cognition loss, often associated with neurological diseases.

An increase in adult neurogenesis can be attained by increasing the production of new neurons or increasing their survival. Survival is an interesting parameter to act on, because most of the adult-born hippocampal neurons (70%) die by apoptosis within the third week of their maturation (Figure 11). This suggests that these adult-born neurons undergo stringent selection, and that increasing their ability to pass this selection will increase their survival, and therefore increase adult neurogenesis. On which criteria is this selection made?

Role of synaptogenesis and synaptic activity in the integration and survival of hippocampal adult-born neurons

The majority of adult-born neurons die before completing their maturation. Kempermann and colleagues injected adult mice with BrdU once a day for 12 consecutive days to label adult-born hippocampal neurons. Mice were sacrified at different timepoints after the last injection of BrdU, and the number of BrdU-labeled cells was counted[24]. In another publication by Zhao *et al.*, the formation of glutamatergic inputs on newborn neurons was analyzed by retroviral-mediated labeling of adult-born neurons: dendritic spine density was counted at different timepoints after retroviral injection[28]. Interestingly, neuronal cell death correlates with the formation of the first glutamatergic inputs on dendritic spines and the first mature synapses with CA3 pyramidal cells (Figure 11). This suggests that synaptogenesis and survival are linked.

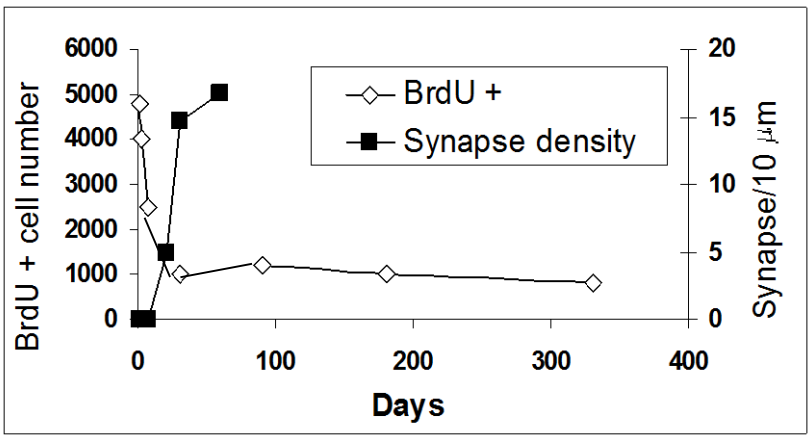


Figure 11: The apoptosis of adult-born hippocampal neurons correlates with the formation of the first glutamatergic synapses. White line: Number of Bromodeoxyuridine (BrdU)-labeled cells at various time-points after BrdU injection. Black line: Excitatory synapse density. BrdU labels cells in division and was used to label adult-born neurons born at the time of injection. Numbers are absolute cell counts per animal. The decrease in BrdU+ cell numbers parallels the increase in excitatory synapse density of adult-born neurons, suggesting that the death of adult-born neurons and excitatory synaptogenesis are linked. Adapted from Kempermann *et al.* 2003 and Zhao *et al.* 2006[24,28].

Secondly, newborn neuron survival seems to depend on neuronal activity: enriched environment increases the survival of newborn granule cells, leading to an overall increase in hippocampal neurogenesis in rodents [46]. Learning can also impact adult neurogenesis: hippocampus-dependent learning tasks increase the survival of hippocampal adult-born neurons, whereas non hippocampus-dependent learning tasks have no effect on this process[76,77].

Thus, activities increasing neuronal activity, such as learning, can increase the survival of hippocampal adult-born neurons.

More compelling evidence for a link between neuronal activity and adult-born neuron survival was provided by studies using genetic modification of adult-born neurons. Tashiro and colleagues showed that a cell-specific knock-out of the NMDA receptor 1 (NR1) subunit of the NMDA receptor in adult-born neurons dramatically decreased newborn neuron survival during the third week after division (Figure 12) [78].

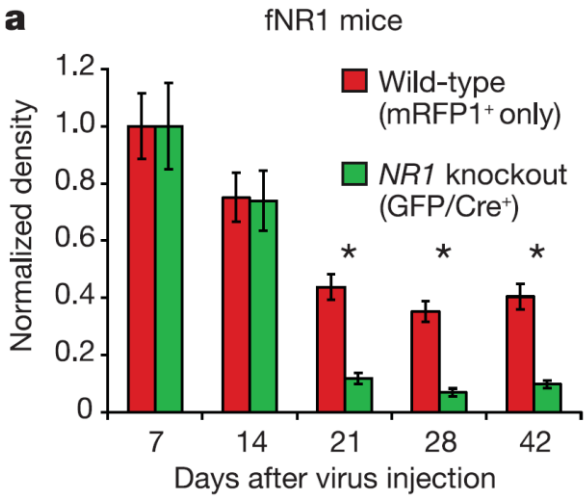


Figure 12: The absence of functional NMDA receptor reduces the survival rate of adult-born neurons. Adult mice floxed for the NR1 subunit of NMDA receptor, i.e. in which NR1 gene is flanked by target sequences of Cre recombinase, were injected with a mix of two retrovirus, one carrying the expression cassette of Red fluorescent protein (RFP), and the other carrying the expression cassette of Cre recombinase fused to the Green fluorescent protein (GFP). As these retroviruses selectively infect neuronal precursor cells, this resulted in two subpopulations of adult-born neurons in the same mice, one expressing RFP, and the other one expressing Cre-GFP and thus knocked out for NR1. The survival rate of NR1 knockout GFP-positive neurons was reduced compared to control RFP-positive neurons. Adapted from Tashiro *et al.* 2006 [78]

Also, Ge and colleagues showed that the experimental conversion of GABA-induced depolarization into hyper-polarization in newborn neurons before three weeks of maturation by knock-down of NKCC1 induces marked defects in synapse formation and dendritic development *in vivo* [36] (Figure 13). Indeed, newborn neurons expressing a shRNA against NKCC1 showed decreased dendritic arborization and decreased dendritic length (Figure 13a). The formation of GABAergic and glutamatergic synapses was also altered (Figure 13c-f). Thus,

neurons in which GABA-induced depolarization was annihilated showed defects in synaptic integration and maturation.

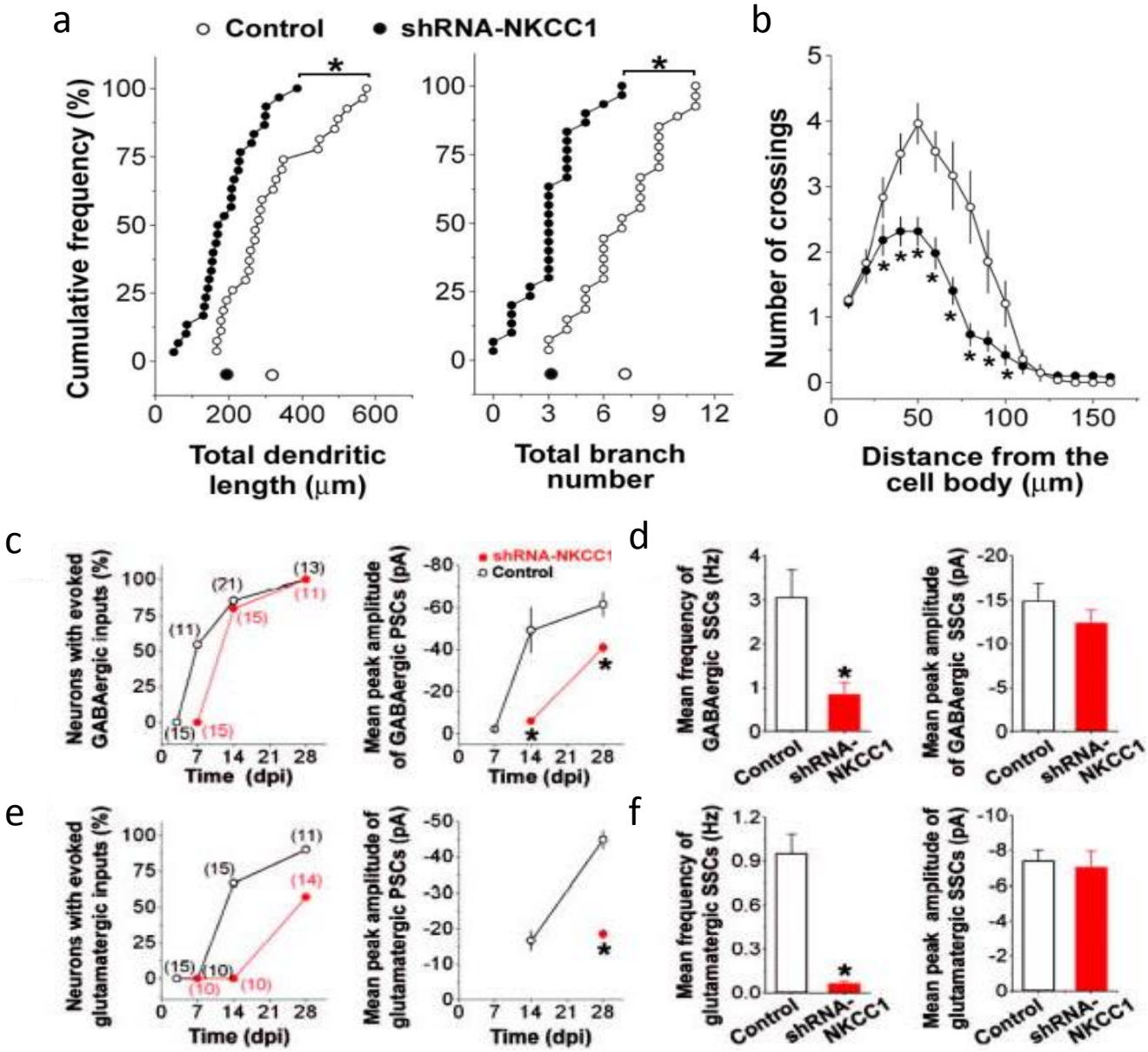


Figure 13: GABA is essential for the proper synaptic maturation and integration of adult-born neurons. a, Quantification of total dendritic length and branch number of Nkcc1-shRNA-expressing and control newborn neurons at 14 dpi. b, Sholl analysis of the dendritic complexity of Nkcc1-shRNA-expressing and control newborn neurons. c-d, Formation of GABAergic synaptic inputs by control and NKCC1-shRNA-expressing neurons. Shown are the percentage of newborn neurons with detectable GABAergic post-synaptic currents (PSCs), mean amplitude of GABAergic PSCs (c), mean frequency and peak amplitude of GABAergic SSCs recorded at 28 dpi (d). Numbers associated with symbols refer to the number of cells examined. e–f, Formation of glutamatergic synaptic inputs by newborn neurons. Same as in (c–d), except that the recordings were carried out in the presence of bicuculline (10 μM). Blue lines indicate the addition of CNQX (50 μM). Scale bars: 10 pA and 40 ms. Adapted from Ge *et al.* 2006 [36]

Last but not least, in the adult rat hippocampus, induction of LTP at medial perforant path-granule cell synapses promotes survival of 1 to 2 week-old adult-born dentate granule cells (Figure 14) [79]. The pseudotetanus (PT) protocol, that does not induce LTP, has no effect on adult-born neuron survival.

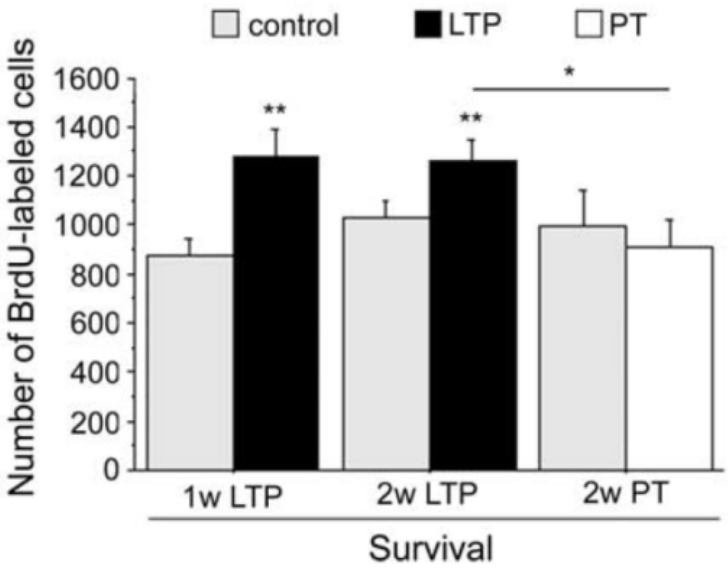


Figure 14: LTP enhances survival of recently born granule cells. Average total number of BrdU-labeled cells in the dentate gyrus from control, and LTP or PT sides. LTP was induced 1 or 2 weeks after BrdU injections and rats were killed at the survival time of 4 weeks post-BrdU. Adapted from Bruel-Jungerman *et al.* 2006 [79]

Therefore, synaptic activity and excitatory as well as inhibitory synaptogenesis may be crucial for neuronal survival and integration in the circuitry. Also, increasing synaptogenesis might increase the survival of hippocampal adult-born neurons and, as a consequence, increase learning and memory.

Experimental part

Most of the adult-born neurons die during their maturation. Acting on adult-born neuronal survival may thus be crucial to influence total neurogenesis and its impact on behavior or

disease. As synaptogenesis and synaptic activity are linked to adult-born neuron survival, increasing synaptogenesis in newborn neurons could increase adult neurogenesis and then increase learning performances.

Which parameters could influence synaptic activity and synaptogenesis of adult-born neurons?

We chose to study three important factors potentially acting on adult-born neuronal synaptic integration and survival. First, we studied the effect of a global increase of GABAergic activity of the network on the development and survival of hippocampal adult-born neurons. Secondly, we examined the development of astrocytic ensheathment on the synapses formed by adult-born neurons and we studied the effect of inhibition of astrocytic glutamate reuptake on the synaptic activity of adult-born neurons, as we hypothesized that astrocytes may be involved in the synaptic integration of newborn neurons. Finally, we examined the role of synaptic adhesion molecules in newborn neurons and studied their cell-autonomous effects on the integration and survival of these neurons.

As a consequence, my PhD project is articulated in three questions:

- **What is the outcome of a global decrease in synaptic activity on the maturation, survival and integration of adult-born hippocampal neurons?**

- **Do astrocytic processes ensheath adult-born neurons synapses, and if so, what is the role of this ensheathment on the synaptic integration of adult-born neurons ?**

- **What are the consequences of an increase in synaptogenesis on the survival and integration of adult-born neurons?**

Mechanisms involved in the synaptic integration of adult-born neurons potentially represent a very efficient way to stimulate adult neurogenesis in the context of neurodegenerative diseases or memory loss. Furthermore, virally-labelled cohorts of newborn neurons with simultaneous synaptogenesis occurring on large dendritic trees represent an ideal model to study the mechanisms of synaptogenesis in the adult brain, such as those occurring during LTP expression, that are normally scarce events.

Project 1: What is the outcome of a global decrease in synaptic activity on the maturation and integration of adult-born hippocampal neurons?

Introduction

The aim of this project is to examine the role of the network activity on the synaptic integration and survival of immature neurons.

Anesthetics modulate GABAergic and glutamatergic transmission, and they decrease the brain excitation/inhibition balance. Anesthesia is therefore an efficient way to globally decrease synaptic activity. Moreover, some experimental work has shown that anesthetics can impair post-natal neurogenesis and induce cognitive impairment: Zhu and colleagues showed in 2010 that repeated isoflurane exposure of juvenile rats impaired object recognition performances, and that the deficits became more pronounced as the animals grew older [80] (Figure 15a-b). This memory deficit was accompanied by a persistent decrease in the neural stem cell pool and cell proliferation, leading to persistently reduced neurogenesis (Figure 15c). Older rats' scores for the same behavioural tests were not affected, and their neurogenesis was only slightly affected by isoflurane exposure (Figure 15a-c).

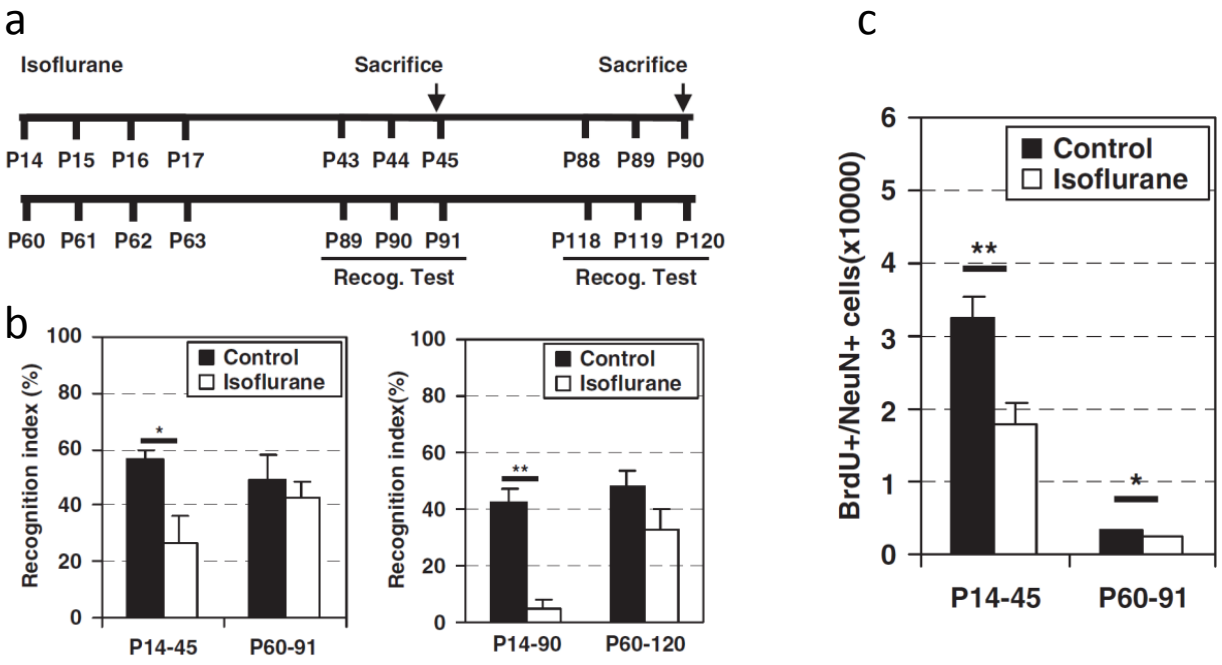


Figure 15: Repeated exposure to isoflurane impairs object recognition in young rats, and the deficits become more pronounced when they grow older. a, Experimental design for the young (post-natal

day 14, P14) and older (P60) rats. The rats were exposed to isoflurane (isoflurane) or a mixture of oxygen and air (control) for 35 minutes daily for four successive days. Object recognition was tested 4 weeks (P43 for young rats, P89 for old rats) or 10 weeks (P88 for young rats, P118 for older rats) after the last isoflurane exposure. b, Recognition memory index was significantly reduced 4 weeks after isoflurane exposure in P14 to P45 rats, but not P60 to P91 rats (left panel). The recognition memory index reduction was even more pronounced 10 weeks after isoflurane exposure in P14 to P90 rats, but not P60 to P120 (right panel). c, Isoflurane decreased adult neurogenesis in both the P14 to P45 and the P60 to P91 rats, but the decrease was more pronounced in P14 to P45 rats. Adapted from Zhu *et al.* 2010 [80]

Moreover, prolonged exposure to anesthetics such as ketamine, propofol or isoflurane induces neuronal apoptosis and neurodegeneration in rodent and monkey developing brains. In ten day-old mice, coadministration of ketamine with propofol or ketamine with thiopental or a high dose of propofol alone triggers neurodegeneration [81] (Figure 16a). Also, exposure of ten day-old mice to a combination of anesthetic agents or ketamine alone resulted in functional deficits in adulthood: adult mice display disrupted learning in a radial arm maze behavioural test (Figure 16b).

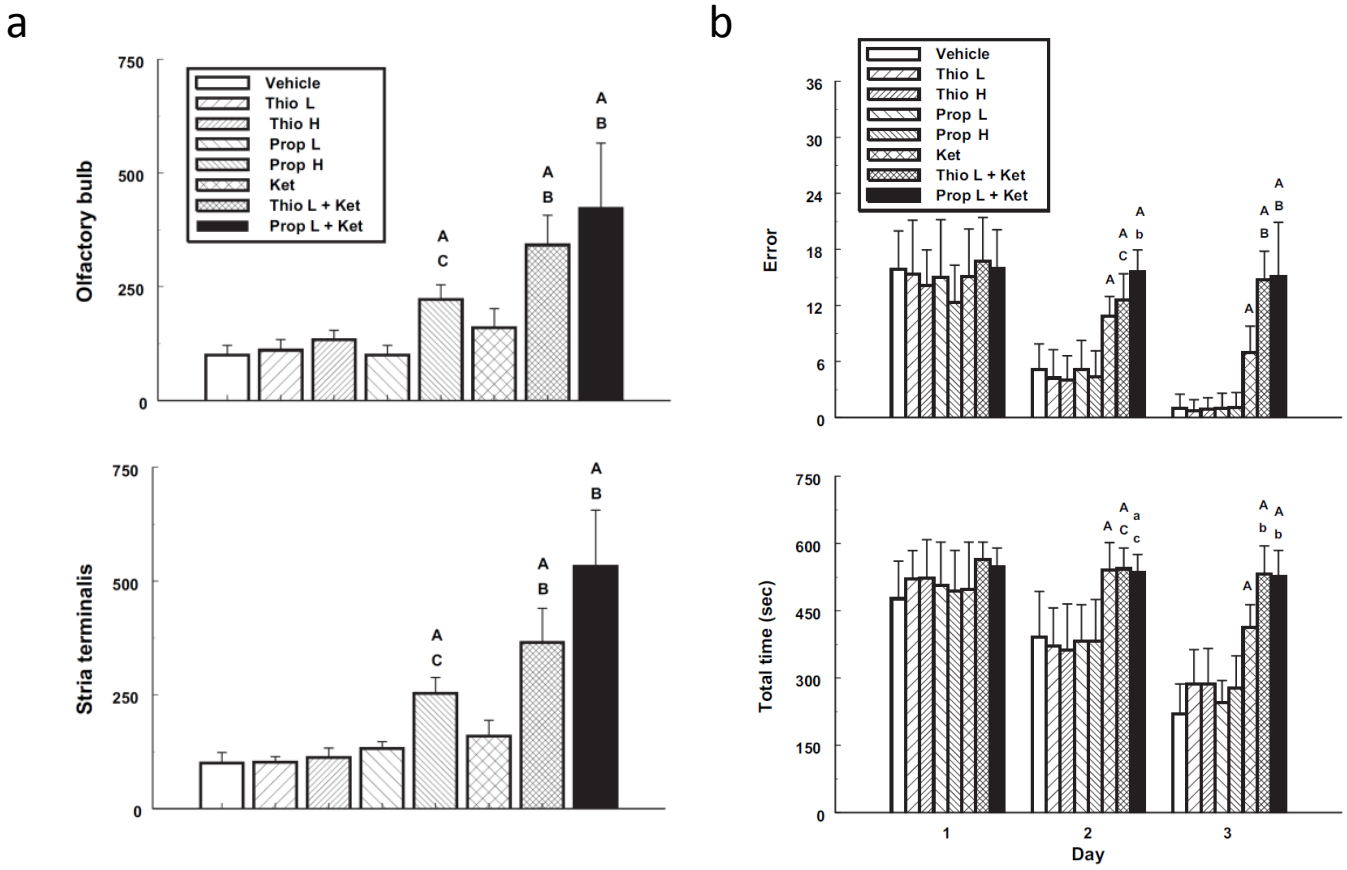


Figure 16: Prolonged exposure of mouse pups to anesthetics induces neurodegeneration and functional deficits in adulthood. a, Fluoro-Jade staining in olfactory bulb (upper panel) and stria terminalis (lower panel) of mouse pups treated with vehicle, 5 mg/kg thiopental (Thio L), 25 mg/kg thiopental (Thio H), 10 mg/kg propofol (Prop L), 60 mg/kg propofol (Prop H), 25 mg/kg ketamine (Ket), 25 mg/kg ketamine plus 5 mg/kg thiopental (Thio L _ Ket) or 25 mg/kg ketamine plus 10 mg/kg propofol (Prop L _ Ket) on neonatal day 10 and killed 24 h later. Staining is expressed as a percentage of vehicle-treated mouse pups. b, Radial arm maze acquisition performance of adult mice treated with the same anesthetics as in a, on neonatal day 10 and tested in the radial arm maze at 63 days of age. Number of errors (upper panel) and total time (seconds, lower panel) on 3 consecutive days of testing. A: significant difference versus vehicle; B: significant difference versus all monotherapy groups; C: significant difference versus Prop L, at 1% level of significance. Letters in uppercase and lowercase represent 1% and 5% levels of significance, respectively. Adapted from Fredriksson et al. 2007 [81]

In PND5 rhesus monkeys, 24-hour exposure to ketamine induced neuronal cell death, whereas 3-hour exposure has no effect [82]. 24-hour exposure to ketamine has no effect on PND 35 animals.

Therefore, anesthetics can impair brain development and subsequently affect learning performances and behavior in adulthood. However, the effects of anesthesia on the adult brain are less clear. As described in the previous chapters, adult-born neurons undergo intense development and their morphological and functional maturation depends on the GABAergic afferences they receive. Therefore, they may be particulary sensitive to anesthesia exposure.

Propofol is an anesthetic commonly used in human surgery, and it exerts its effect primarily by potentiating GABAergic transmission. In this part of the project, we tested whether potentiating global GABAergic transmission during 6 hours with propofol anesthesia would affect neuronal development and survival in adult mice.

Results

The results of this project were published in the following article:
Marine Krzisch, Sébastien Sultan, Julie Sandell, Kornél Demeter, Laszlo Vutskits, Nicolas Toni (2013) **Propofol Anesthesia Impairs the Maturation and Survival of Adult-born Hippocampal Neurons**, *Anesthesiology*

We found in this article that adult-born mouse hippocampal neurons are vulnerable to propofol anesthesia, which reduced neuron survival and dendritic maturation *in vivo*. Propofol impaired the survival and maturation of adult-born neurons in an age-dependent manner. Anesthesia induced a significant decrease in the survival of neurons that were 17 days old at the time of anesthesia, but not of neurons that were 11 days old. Similarly, propofol anesthesia significantly reduced the dendritic maturation of neurons generated 17 days before anesthesia, without interfering with the maturation of neurons generated 11 days before anesthesia. These results reveal that propofol impairs the survival and maturation of adult-born hippocampal neurons in a developmental stage-dependent manner in mice.

Contribution to the project

I produced the virus used in this study. I performed retroviral labeling of adult-born neurons by injecting a retrovirus carrying GFP expression cassette in the dentate gyrus of adult C57BL6/J mice. Then, I anesthetized the mice, sacrified them, immunostained the slices, took confocal micrographs and performed morphological analyses (Figures 2 and 3 of the article). To study control neurons, DiI labeling and analysis of DiI labeling data were performed by Julie Sandell, whereas microinjections were performed by Kornél Demeter and data from the microinjections were analyzed by myself. I contributed to the writing of the article.

Project 2: Do astrocytic processes ensheath adult-born neurons synapses, and is this ensheathment functionally relevant?

Introduction

The aim of this project is to examine the role of cellular contacts between immature neurons and astrocytes in the synaptic integration of adult-born neurons.

Astrocytes may have a fundamental importance during the development of adult-born neurons. Astrocytes extend processes that make contact with most neuronal synapses in the central nervous system (CNS) (Figure 17) [83,84].

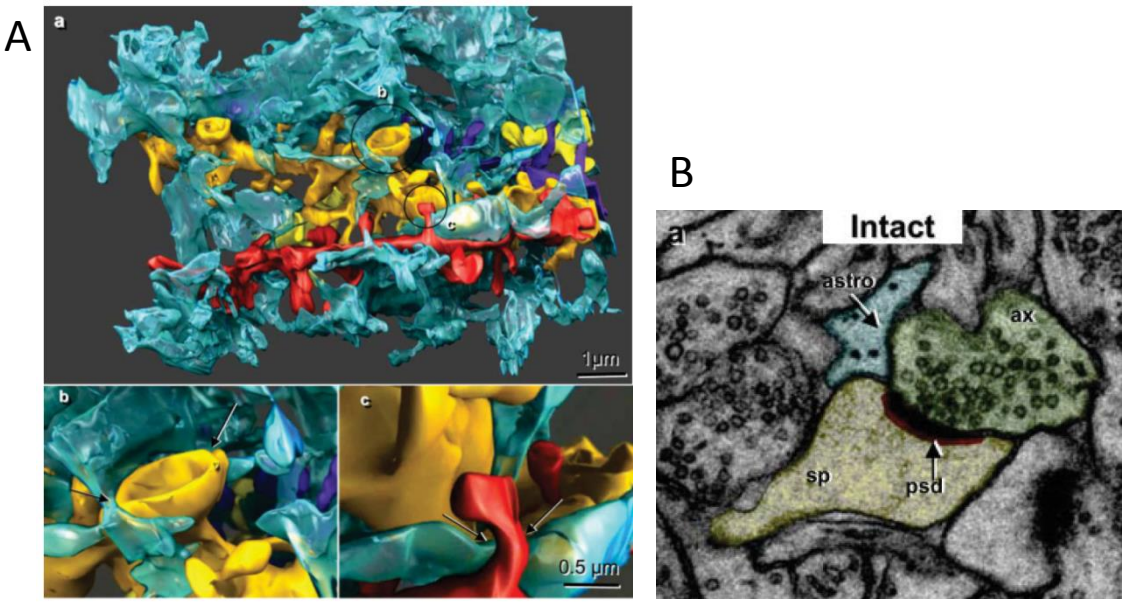


Figure 18: Astrocytic processes ensheath synapses in the central nervous system. A a, Three-dimensional reconstruction of a single astroglial process (blue) interdigitating among many dendrites, four of which are reconstructed here (gold, yellow, red, and purple). Axonal boutons are not displayed. b, Mushroom spine apposed by astroglia (arrows). c, Thin dendritic spine apposed by astroglia (arrows). Scale bar in (c) is for (b) and (c). B, Astroglial processes at the axon-dendritic spine interface; astro, astroglial process (blue); psd, postsynaptic density (red); sp, dendritic spine head (yellow); ax, axonal bouton (green). Adapted from Witcher *et al.* 2007 [83]

These processes have a crucial role in synapse function and plasticity [84]. Furthermore, astrocytic processes reuptake glutamate from the synaptic cleft, and this is crucial for synaptic function, but also for neuronal survival. Rothstein and collaborators used antisense oligonucleotides to chronically inhibit the synthesis of astroglial glutamate transporters in adult rats. The loss of

astroglial glutamate transporters GLT-1 or GLAST in rat resulted in elevated extracellular glutamate levels and neurodegeneration characteristic of excitotoxicity. Thus, glial glutamate transporters are essential for maintaining low extracellular glutamate and preventing chronic glutamate neurotoxicity [85].

Astrocytes also play a crucial role in synapse formation and maturation during embryonic development. Synaptogenesis and astrocytogenesis occur simultaneously [86,87], and astrocytes express cell membrane-bound and soluble factors enabling synaptic maturation and stability.

In 2001, Ullian and colleagues showed that in the absence of glial cells, the number of synapses formed by retinal ganglion neurons in culture decreased by sevenfold and that the few synapses that did form were functionally immature: the size of the postsynaptic response of neurons without glia was decreased by three folds (Figure 19) [88]. Thus, glia increases synapse number and efficacy.

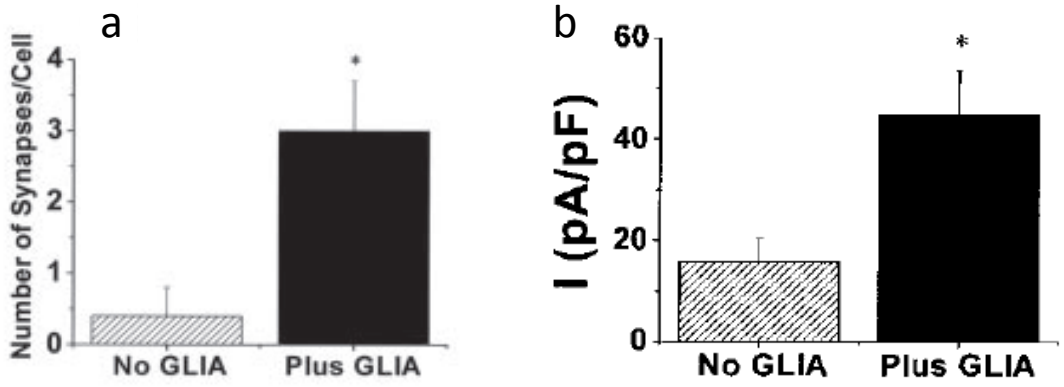


Figure 19: Astrocytes increase the number of synapses per neuron. a, The number of synapses formed by retinal ganglion neurons in culture increased sevenfold in the presence of glia. Synapses were detected by electron microscopy. b, Current densities of retinal ganglion cells cultured in the absence or presence of glia. Adapted from Ullian *et al.* 2001 [88]

In 2005, Christopherson and colleagues confirmed that astrocytes promote excitatory synaptogenesis and synapse maturation *in vivo*: they showed that immature but not mature astrocytes expressed thrombospondins-1 and -2 and that these proteins promoted synaptogenesis *in vitro* but also *in vivo*: TSP1/2 double knock-out mice cerebral cortex displayed decreased synapse number (Figure 20) [89].

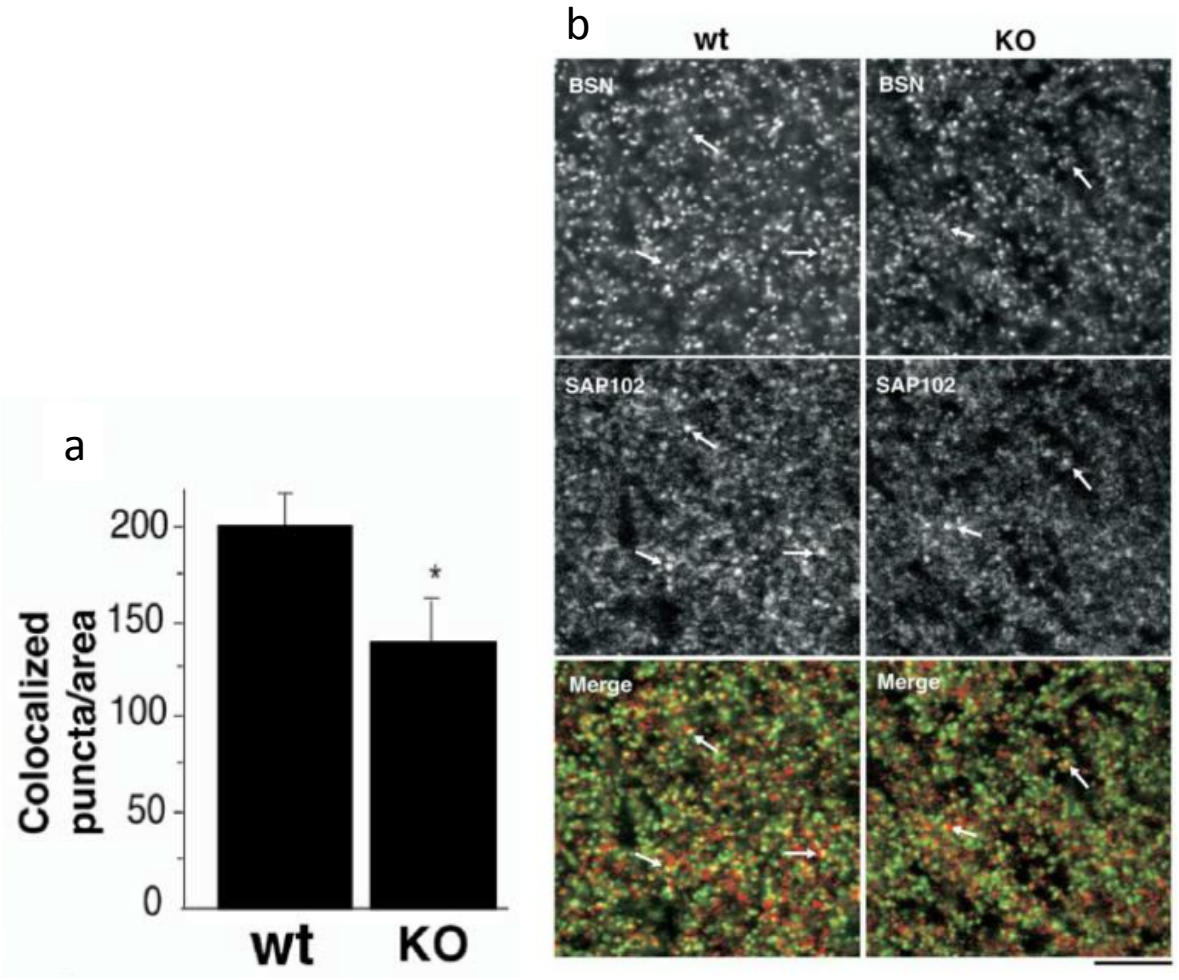


Figure 20: The cerebral cortex of TSP1/2 double knockout mice displays decreased synapse number compared to control. a, Quantification of colocalization of pre- and postsynaptic markers in brain sections from wild-type and TSP1/2 P21 double knockout brains. b, Wild-type and TSP1/2 double null P21 cortex immunolabeled for presynaptic Bassoon (BSN) and postsynaptic SAP102. Fewer synaptic puncta containing both pre- and postsynaptic markers are present in TSP1/2 double knockout brains compared to wild-type. Scale bar is 6 µm. Adapted from Christopherson *et al.* 2005 [89]

Therefore, astrocytes may play a very important role in the synaptogenesis of adult-born neurons, and thus in their integration and survival. However, shortly after birth, gliogenesis is reduced [90], and the role of astrocytes in synaptogenesis occuring in the adult brain is not clear [83]. Notably, is unknown whether astrocytes interact with synapses formed by adult-born neurons and participate to their function.

Astrocytes remain plastic throughout adulthood [83,91-93]. In particular, Haber and colleagues showed that astrocytes can rapidly extend and retract their processes to engage and disengage from motile dendritic spines [94]. Furthermore, astrocytic motility is, on average, even higher than its dendritic spine counterparts (Figure 21).

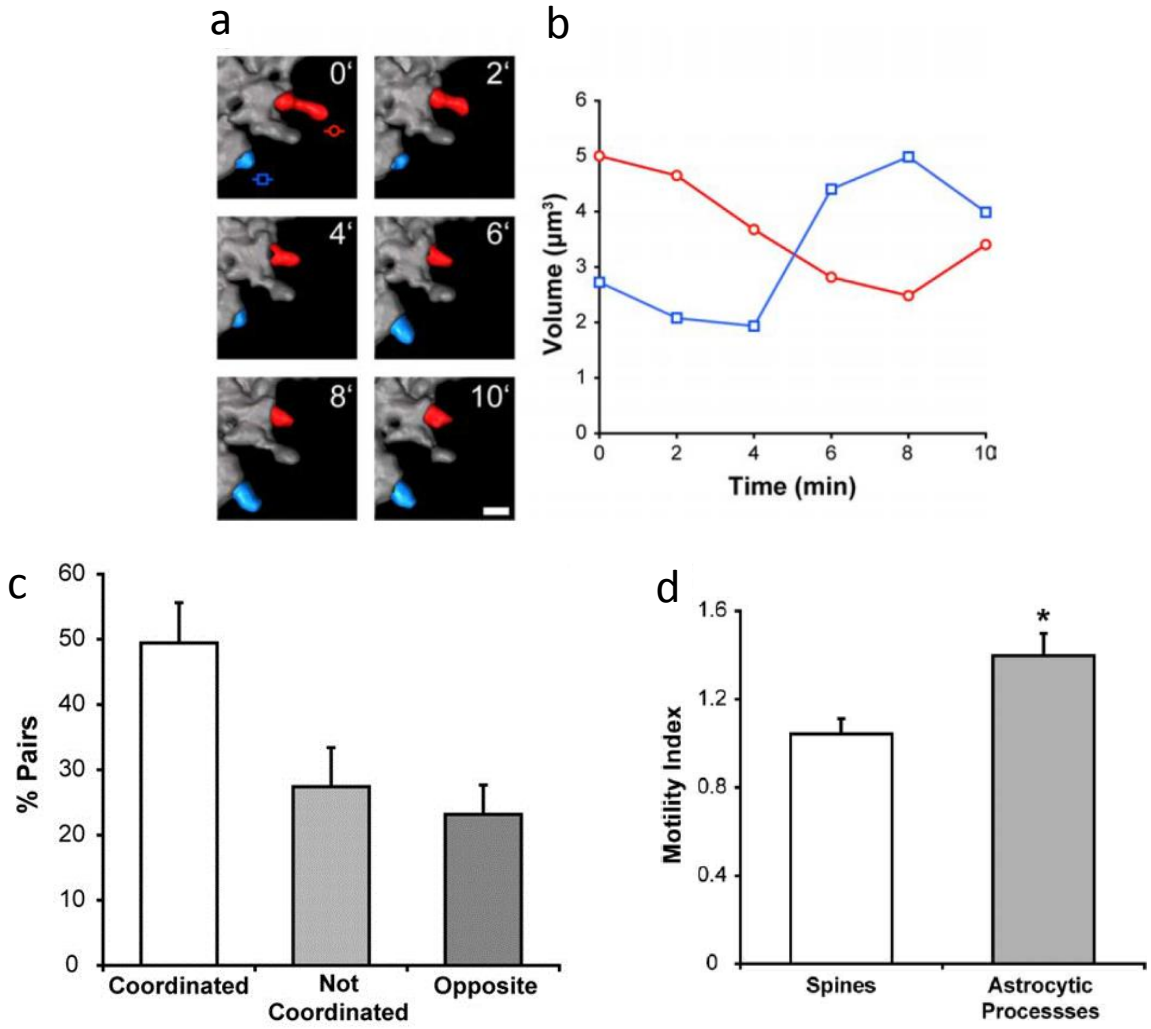


Figure 21: Astrocytic processes undergo rapid structural modifications, typically coordinated with structural modifications of dendritic spines. a, Three-dimensional reconstructions of astrocytic processes over a 10 min period. b, Volume measurements of retracting (red circles, corresponding to the red process in a) and extending (blue squares, corresponding to the blue process in b) processes plotted over time. Scale bars: a, 10 µm; b, 4 µm. c, Approximately 50% of astrocytic process–spine pairs show coordinated changes in size over time, 27% are not coordinated, and 23% show opposite changes. d, Astrocytic processes have significantly higher motility indices than spines.

Our hypothesis is that astrocytic perisynaptic processes participate to the establishment and plasticity of synapses formed by adult-born neurons. However, although astrocytes remain plastic throughout adulhood, the extensive synaptogenesis occurring during the maturation of adult-born neurons may not be accompanied by the simultaneous generation of astrocytic processes from pre-existing astrocytes. Therefore, as a first step, we studied the development of astroglial perisynaptic processes on the dendritic spines of neurons born in the adult hippocampus and the role of astrocytic glutamate transporters in their synaptic transmission.

Results

The results of this project were published in the following article:

Marine Krzisch, Silvio G. Temprana, Lucas A. Mongiat, Jan Armida, Valentin Schmutz, Mari A. Virtanen, Jacqueline Kocher-Braissant, Rudolf Kraftsik, Laszlo Vutskits, Karl-Klaus Conzelmann, Matteo Bergami, Fred H. Gage, Alejandro F. Schinder, Nicolas Toni (2014) **Pre-existing astrocytes form functional perisynaptic processes on neurons generated in the adult hippocampus,** *Brain structure and function*

We found that the afferent and efferent synapses of newborn neurons are ensheathed by astrocytic processes, irrespective of the age of the neurons or the size of their synapses. The quantification of gliogenesis and the distribution of astrocytic processes on synapses formed by adult-born neurons suggest that the majority of these processes are recruited from pre-existing astrocytes. Furthermore, the inhibition of astrocytic glutamate re-uptake significantly reduced postsynaptic currents and increased paired-pulse facilitation in adult-born neurons, suggesting that perisynaptic processes modulate synaptic transmission on these cells. Finally, some processes were found intercalated between newly formed dendritic spines and potential presynaptic partners, suggesting that they may also play a structural role in the connectivity of new spines. Together, these results indicate that pre-existing astrocytes remodel their processes to ensheathe synapses of adult-born neurons and participate to the functional and structural integration of these cells into the hippocampal network.

Contribution to the project

I analyzed all the electron microscopy data produced by Nicolas Toni, and I made the three-dimensional reconstructions presented in Figure 2, 3 and 5 (except 3F, which was made by Nicolas Toni). I maintained transgenic mouse lines (Aldh1l1-GFP and GFAP-GFP mice). I produced a virus carrying the RFP expression cassette and injected it in the dentate gyrus of adult mice expressing GFP under the astrocytic promoter Aldh1l1 or GFAP (Aldh1l1-GFP and GFAP-GFP mice) to label newborn neurons. I sacrified mice at different timepoints and performed confocal imaging and analyses of the dendritic spines and of the arborization of adult-born neurons. I supervised a master student, Jan Armida, who took confocal micrographs of newborn neuron mossy fiber terminals in hippocampal slices from the mice I injected and analyzed them (Figure 2 and Supplementary figure 3). Figure 3A and 3B were obtained using hippocampal slices from mice injected with BrdU by Sebastien Sultan. I supervised a summer student, Valentin Schmutz, who produced the data displayed in Supplementary figure 2. I contributed to the writing of the article.

Project 3: What are the consequences of an increase in synaptogenesis on the survival and integration of adult-born neurons?

Introduction

The aim of this project is to enhance synaptogenesis on new, immature neurons, in a cell-autonomous manner and to examine the effect of this manipulation on the integration and survival of newborn neurons.

Neuronal cell adhesion molecules are proteins located on the neuronal cell surface, involved in binding with other cells or with the extracellular matrix. Previous work showed that some of these molecules are able to increase synaptogenesis and synapse maturation in neurons from the CNS. Synaptic Adhesion Molecule 1 (SynCAM1) is a transmembrane protein located pre- and post-synaptically, and forms homo- or heterodimers with the other members of the SynCAM1 family [95,96]. Biederer and colleagues were the first to show that SynCAM1 is involved in excitatory synaptogenesis [95]. SynCAM1 expressed in non-neuronal cells induced co-cultured hippocampal neurons to form presynaptic specializations onto these cells. Moreover, SynCAM1 overexpression increased the frequency of spontaneous miniature synaptic currents in co-cultured hippocampal neurons. As miniature frequency depends primarily on the number of synapses and their release probability, the authors concluded that SynCAM1 induced synapse formation in these neurons.

SynCAM1 contains an NH2-terminal signal peptide, three extracellular Ig domains, a single transmembrane region, and a short COOH-terminal cytoplasmic tail. SynCAM1 mutant SynCAMΔIg, that lacks Ig domains and is unable to undergo extracellular homophilic interactions, had no effect on synapse formation. Thus, SynCAM1 extracellular interactions are necessary for its function. As a consequence, the isolated cytoplasmic tail of SynCAM1 competes with SynCAM1 for binding to its cytosolic substrates, and as it cannot induce synapse formation, is expected to act as a dominant negative fragment by inhibiting endogenous SynCAM1. As expected, the isolated cytoplasmic tail of SynCAM1 acted as a dominant negative form of SynCAM1 and inhibited synapse assembly [95].

In 2010, the Robbins and colleagues showed that overexpression of SynCAM1 in excitatory neurons of the forebrain of adult mice specifically increased excitatory synapse number (Figure 22) in the CA1 region of the hippocampus. SynCAM1 knock-out mice showed the opposite phenotype: excitatory synapse density was decreased in the CA1, whereas inhibitory synapse density was unchanged (Figure 22) [97].

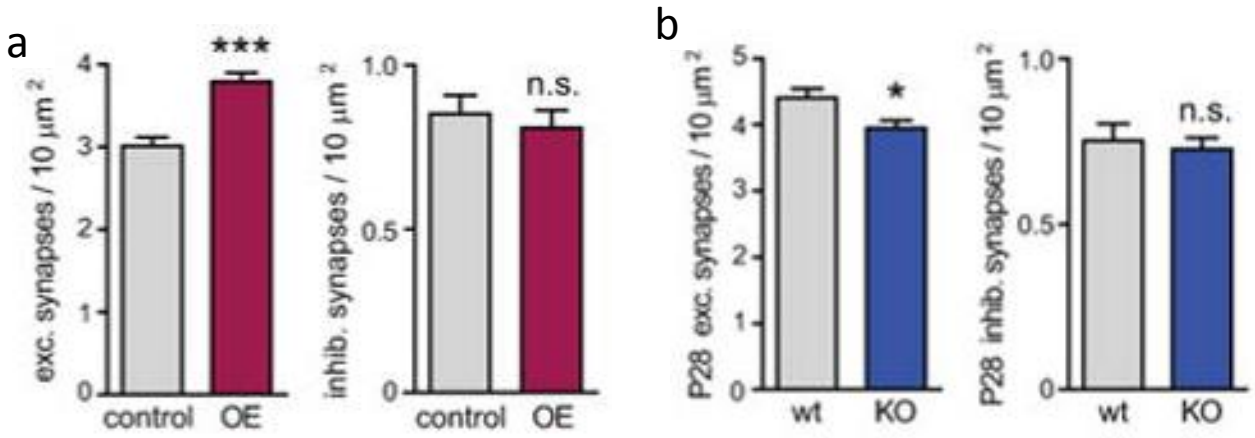


Figure 22: SynCAM1 Regulates Excitatory Synapse Number. a, SynCAM1 overexpression increases excitatory synapse density (left panel). Inhibitory synapse density is unaffected by overexpression (right panel). b, Lack of SynCAM1 reduces excitatory synapse number (left panel) but does not affect inhibitory synapse density (right panel). Adapted from Robbins *et al* 2010 [97]

Similarly, Sara and colleagues showed that SynCAM1 overexpression increased the frequency of spontaneous miniature synaptic currents in cultured hippocampal neurons [98]. However, morphological analysis of neurons overexpressing SynCAM1 showed the opposite of the predictions from electrophysiological analyses: SynCAM1 overexpression did not increase excitatory synapse number. Therefore, in this study, SynCAM1 specifically increased excitatory synapse function, without having an effect on synapse number. This contradicts Biederer's *in vivo* findings, and may be explained by the overexpression of SynCAM1 in all the excitatory neurons of the forebrain in Biederer's study. We indeed cannot exclude that the increase in excitatory synapse density in CA1 neurons observed by Biederer *in vivo* is due to a non-cell- autonomous effect of SynCAM1 overexpression on CA1 neurons.

Neuroligin-1 (NL1) and Neuroligin-2 (NL2) are two members of a family of neuronal cell adhesion molecules, the neuroligins. Neuroligins are transmembrane proteins located post-synaptically, and act as ligands for neurexins, located presynaptically. Numerous studies have shown that neuroligins mediates the formation and maintenance of synapses between neurons *in vitro* and *in vivo* [99-103]. Initial evidence came from Scheiffele and colleagues, who demonstrated that both NL1 and NL2 expressed in a non-neuronal cell could induce co-cultured neurons to form presynaptic specializations onto the non-neuronal cell. This finding was amplified by complementary experiments showing that β-neurexin, that binds NL1 and NL2, can induce post-synaptic specializations in co-cultured neurons when expressed in a non-neuronal cell. Finally, direct overexpression of NL1 in transfected neurons caused an increase in synapse numbers on these neurons [104,105] (Figure 23).

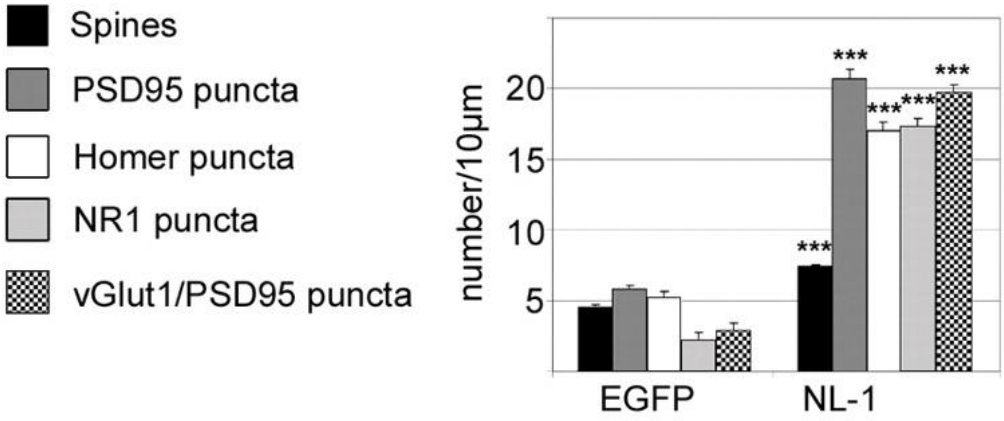


Figure 23: NL1 promotes synaptic differentiation. Hippocampal neurons were co-transfected with expression vectors for NL-1 and GFP or with GFP vectors only. Quantification of post-synaptic protein recruitment, dendritic spine induction, and synapse formation in cells expressing NL-1 and EGFP-transfected control cells. Adapted from Chih *et al.* 2005 [105]

Interestingly, NL1 and NL2 are alternatively spliced in their ectodomains, i.e. domains that extend into the extracellular space. Inclusion of short alternative exons at two sites, named inserts A and B, generates four NL1 variants: NL1(-) (without insert), NL1A (with insert A), NL1B (with insert B), and NL1AB (with both inserts). NL1B and NL1AB are abundant, whereas only a small pool of NL1(-) and NL1A is present in hippocampal neurons [106] (Figure 24). NL2 undergoes a similar splicing event at the first site, generating two splice variants: NL2A and NL2(-). NL2A is the most abundant variant in hippocampal neurons. This alternative splicing controls the synapse-inducing activity of NL1 and NL2 toward glutamatergic and

GABAergic axons. In cultured hippocampal neurons, NL1(-) and NL1A significantly increase the density of both glutamatergic and GABAergic synapses, whereas NL1B and NL1AB greatly increase excitatory glutamatergic synapse density but have little effect on GABAergic synapses. In contrast, NL2A and NL2(-) show similar induction of glutamatergic and GABAergic synapses when overexpressed [106].

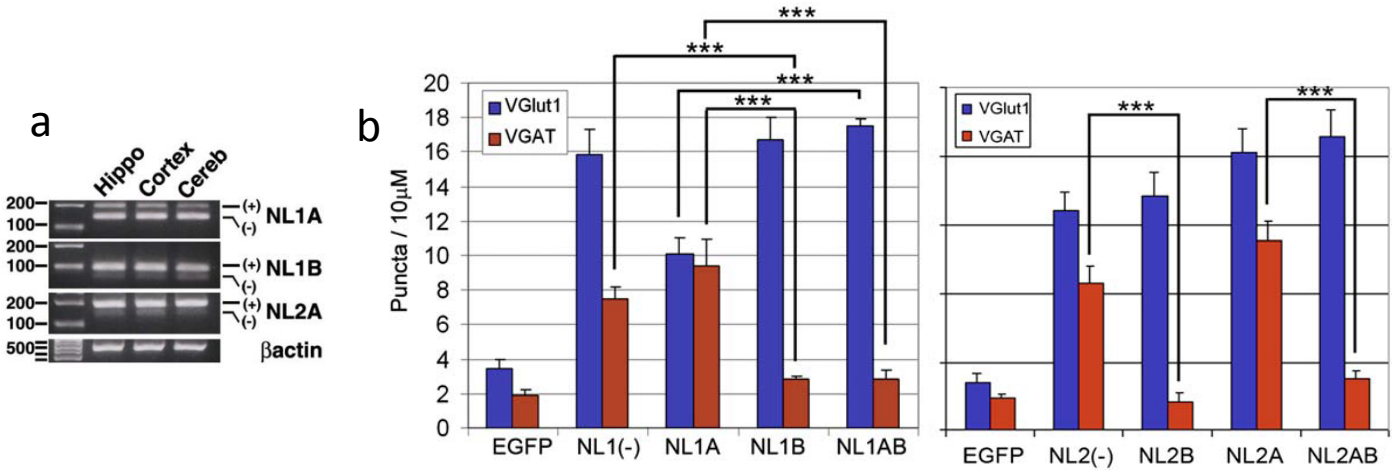


Figure 24: Alternative splicing controls the synapse-inducing activity of NL1 and NL2 toward glutamatergic and GABAergic axons. a, RT-PCR analysis with primers flanking the splice insertion sites A and B on RNA isolated from rat hippocampus, cortex, and cerebellum. β-actin was amplified as a positive control. Products containing (+) and lacking (−) the splice insertion A or B are marked. Molecular weight markers are in base pairs. b, Density of presynaptic vGlut1- and VGAT-positive puncta on cells overexpressing EGFP, NL1(−), NL1A, NL1B, or NL1AB. c, Density of presynaptic vGlut1- and VGAT-positive puncta on cells overexpressing EGFP, NL2(−), NL2B, NL2A, or NL2AB. Adapted from Chih *et al.* 2006 [106]

We focused our study on four molecules: SynCAM1, SynCAM1 cytosolic tail (dominant negative form of SynCAM1), NL1B and NL2A. SynCAM1, NL1B and NL2A were expected to act on different aspects of synaptogenesis: SynCAM1 would specifically increase excitatory synapse number and/or strength, NL1B would specifically increase excitatory synapse formation, and NL2A would increase both excitatory and inhibitory synapse formation. We hypothesized that synaptogenesis and neuronal survival are linked because studies suggest that neuronal survival is activity-dependent, and because synaptogenesis and neuronal death occur during the same time window. We expected these different adhesion molecules to modify different aspects of newborn neuron synaptogenesis. This way, we could distinguish what aspects of synaptogenesis were important for neuronal survival and maturation. We

overexpressed these molecules in hippocampal adult-born neurons using a retroviral approach and studied their effect on the maturation and survival of these neurons.

Material and methods

Experimental animals

The animals used in this study were 7- to 9-week-old C57BL6/J male mice. Mice were group-housed in standard cages under light- (12 h light/dark cycle) and temperature-controlled (22°C) conditions. The maximal number of mice per cage was 5. Food and water were available ad libitum. Every effort was made to minimize the number of animals used and their suffering. Experimental protocols were approved by the Swiss animal experimentation authorities (Service de la consommation et des affaires vétérinaires, Epalinges, Switzerland, Authorization number: 2302).

Virus-mediated gene overexpression

The overexpression of the genes of interest was first tested in vitro: we infected Human embryonic kidney (HEK) 293T cells with 10^4 pfu (1µL) of retrovirus carrying the expression cassette of the gene (cag-SynCAM1-IRES-RFP, cag-NL1B-IRES-RFP, cag-GFP-2A-NL2A or cag-GFP-2A-dnSynCAM1), and performed immunohistochemistry against the adhesion molecule (SynCAM1, NL1B, NL2A, or the cytoplasmic tail of SynCAM1, i.e. dnSynCAM1). Expression of the proteins was effective and localized at the cell plasma membrane (SynCAM1, NL1B, NL2A) or in the cytosol (SynCAM1 cytosolic tail), showing proper addressing of the proteins after synthesis (Figure 25).

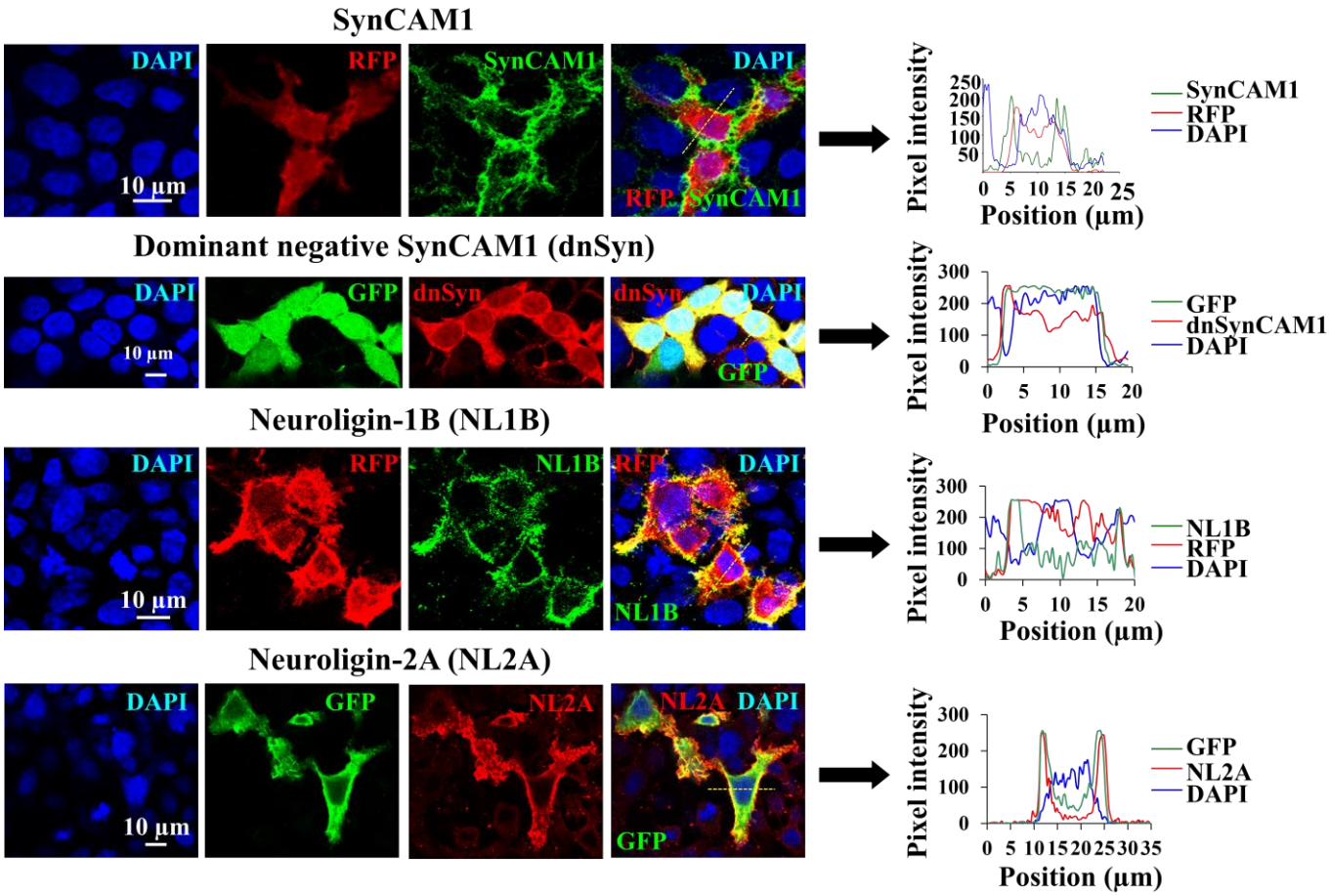


Figure 25: Retroviral infection leads to the expression of the gene of interest and to the proper addressing of the proteins of interest in HEK 293T cells. Left panel: pictures of a single confocal plan from cell cultures infected with the viruses carrying the different constructs. Right panel: plot profiles of the different channels. Expression of the gene was effective. As expected, SynCAM1, NL1B and NL2A proteins were localized at the cell plasma membrane, whereas dnSynCAM1 was cytosolic. Yellow dashed lines show the lines used to build the plot profile.

To selectively overexpress the genes of interest in adult-born hippocampal neurons, we used Moloney murine leukemia viruses (MoMuLV). These retroviruses selectively infect neuronal precursor cells, which give rise to adult-born neurons. Moreover, their half-life is limited to few hours, allowing the overexpression of the genes of interest in a subpopulation of neurons all generated at the same time. Thus, at 14 days post-injection, all labeled neurons are 14 day-old. Also, as the retroviral infection of newborn neurons is sparse, non-cell autonomous effects of the genes of interest can be excluded, because genetically modified granule cells are located far from each other.

Final virus titers were 10^7-10^8 particles forming unit/milliliter (pfu/mL) and 2 µL of the mix were injected into each of the two dentate gyri at the following coordinates from the Bregma: anteroposterior -2 mm, lateral +1.75 or -1.75 mm and dorsoventral -2.25 mm. After every injection and throughout the experiment, animals were regularly monitored for their physical recovery in agreement with, and under the approval of the European and German guidelines on animal experimentation.

To overexpress SynCAM1 or NL1B in adult-born hippocampal neurons, the dentate gyrus of adult mice was injected with a mixture of two MoMuLV, one carrying cag-GFP expression cassette and the other one carrying either cag-SynCAM1 IRES-RFP or cag-NL1B-IRES-RFP expression cassette. To overexpress NL2A or dnSynCAM1 in adult-born hippocampal neurons, the same approach was used with a mix of a virus carrying cag-RFP expression cassette and a virus carrying cag-GFP-2A-NL2A or cag-GFP-2A-dnSynCAM1. Mice were sacrified at different timepoints after injection, corresponding to different developmental stages of newborn neurons: 14 days post-injection (dpi), 21 dpi or 28 dpi. Neurons were imaged with confocal microscopy, and changes their maturation, synaptic integration and survival were analyzed (Figure 26).

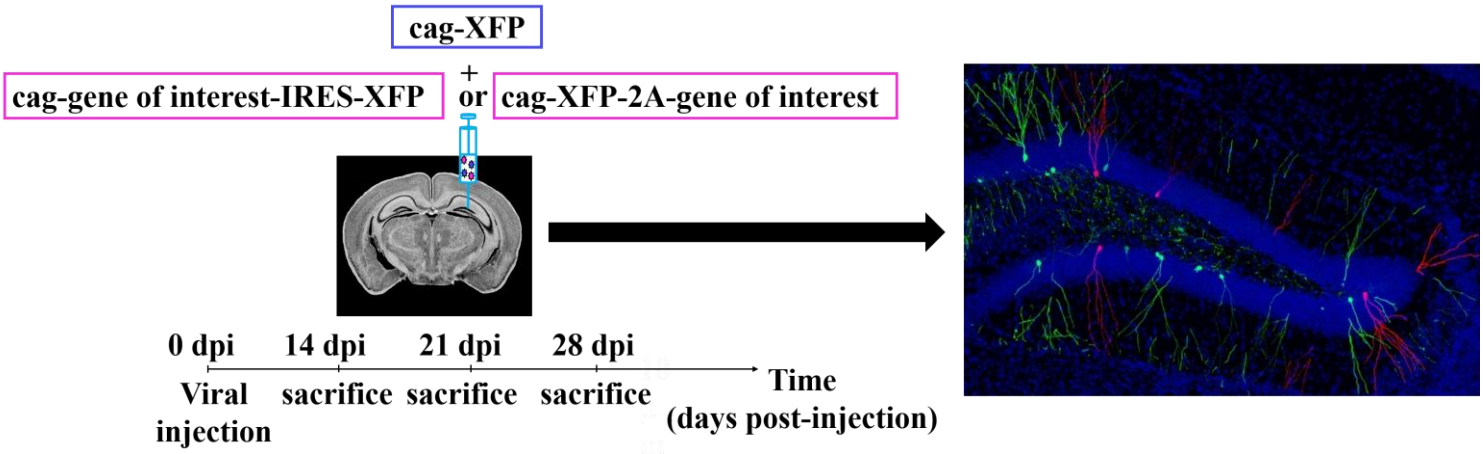


Figure 26: Experimental design. To overexpress the gene of interest in adult-born hippocampal neurons, the dentate gyrus of adult mice was injected with a mixture of two retroviruses, one carrying the expression cassette of RFP or GFP (control retrovirus), and one carrying the expression cassette of the gene of interest and of RFP or GFP (cag-gene of interest-IRES-XFP or cag-XFP-2A-gene of interest). This gave rise to two subpopulations of adult-born granule cells, one expressing the gene of interest and the reporter gene, labeled in one color (either green or red, depending on the constructs), and the other one only expressing the reporter gene and labeled in another color (red or green). Mice were sacrified at different timepoints after injection to study the morphological development of newborn

neurons (lower panel). The picture on the left represents the hippocampus of an adult mouse injected with a mixture of two retroviruses, one carrying the expression cassette of RFP, and the other one carrying the expression cassette of GFP-2A-NL2A.

Brain slice preparation and immunohistochemistry

At 14, 21, and 28 days after viral injection, mice were perfused with 4% paraformaldehyde in phosphate buffered saline, their brains were cryoprotected and sectioned at a thickness of 60 µm. RFP signal was amplified using rabbit anti-RFP IgG (600-401-379 Rockland Immunochemicals, Gilbertsville, Pennsylvania, USA; diluted 1:1000 in phosphate buffer saline containing Normal serum at 3% and Triton at 0.25%) and Hylite 594 goat anti-rabbit IgG secondary antibody (61056-1-H594 Anawa trading SA, Wangen, Switzerland;1:500). GFP signal was amplified using Chicken anti-GFP IgG (GFP-1020 Aves Labs, Tigard, Oregon, USA; 1:1000) and Dylight 488 goat anti-chicken IgY (603-141-126 Anawa Trading SA; 1:500). Vesicular GABA Transporter (VGAT) was detected using mouse anti-VGAT IgG (131011 Synaptic Sytems GmbH, Goettingen, Germany; 1:1000) and Hylite 594 goat anti-mouse IgG secondary antibody (61057-05-H594 Anawa trading SA; 1:500). GABAA receptors were detected using rabbit anti-GABAA receptor IgG (AGA-001 Alomone Labs, Jerusalem, Israel; 1:500) and donkey Dylight 649 anti-rabbit IgG secondary antibody (611-743-127 Anawa trading SA; 1:500).

In HEK 293T cells cultures infected with MoMuLVs, SynCAM1 was detected using chicken anti-SynCAM IgG (CM004-3 MBL International, Woburn, MA, USA; 1:1000) and Dylight 488 goat anti-chicken IgY (603-141-126 Anawa Trading SA; 1:500). dnSynCAM1 was detected using rabbit anti-SynCAM IgG (S 4945, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland; 1:5000) and Cy3 goat anti-rabbit IgG secondary antibody (111-165-144, Jackson ImmunoResearch Laboratories, Westgrove, PA, USA;1:500). NL1B was detected using goat anti-Neuroligin-1 IgG (ANR-036; Alomone labs; 1:25) and donkey Alexa 555 anti-goat IgG secondary antibody (A21432 Invitrogen, Lucerne, Switzerland; 1:250). NL2A was detected using rabbit anti-Neuroligin-2 IgG (sc-50394 Santa Cruz biotechnologies; 1:100) and goat Hylite 594 anti-rabbit IgG secondary antibody (61056-1-H594 Anawa trading SA; 1:500). 4,6 Diamidino-2-phenylindole (DAPI) was used to reveal nuclei.

Confocal microscopy and image analyses

Hippocampal sections were imaged using a Zeiss LSM 710 confocal microscope (Carl Zeiss, Feldbach, Switzerland). Global views of neurons were imaged with a 40x oil lens and a z- step of 2 µm, and dendrites or mossy fiber terminals were imaged with a 63x oil lens and a z-step of 0.38 µm. To assess GABAergic synapse density, dendrites were imaged with a 40x oil lens and a z-step of 0.45 µm. To measure differences in axonal length between dnSynCAM1-expressing and control neurons, the beginning and the end of the CA3 area were imaged using a 40x oil lens and a z-step of 2 µm. All analyses were performed using Fiji software (freely available at http://fiji.sc/).

Dendritic spine density was measured as the number of spines divided by the length of the dendritic segment. Dendritic spine density and diameter were measured on maximal intensity projections. GABAergic synapses were defined as appositions of vesicular GABA transporter (VGAT)-positive and $GABA_A$ receptor ($GABA_AR$)-positive puncta after immunohistochemistry against VGAT and $GABA_AR$. GABAergic synapse density was analyzed on z-stacks and measured as the number of GABAergic synapses divided by the length of the dendritic segment.

The maturation of mossy fiber terminals (MFT) was evaluated following four criteria: area, perimeter, number of extensions and circularity. Circularity was defined as 4 x pi x area / $(perimeter)^2$. During MFT maturation, their area, perimeter and number of extensions increase, whereas their circularity decreases [42,107]. The area and perimeter of mossy fiber terminals were measured on maximal intensity projections by tracing the contour of the MFT, excluding the satellites and filopodia. The number of extensions of the MFT was defined as the number of filopodia or satellites starting at the core of the MFT. Branching of satellites was not included in the counting (Figure 32b).

To measure differences in axonal length between dnSynCAM1-expressing and control neurons, we counted the number of mossy fiber terminals in two areas of the same size: one at the beginning of the CA3 region, and the other one at the end of the CA3 region. We then calculated the ratio between the number of overexpressing mossy fiber terminals and the number of control mossy fiber terminals. If overexpressing axons reached more often the end of the CA3 region, the ratio was expected to increase between the beginning and the end of the CA3. Inversely, if overexpressing axons reached less often the end of the CA3 region, we expected the ratio to

decrease. If there was no difference between control and overexpressing axons, the ratio was expected to remain constant.

Sholl analysis was performed on maximal intensity projections. The space between each radius was set up at 10% of the length of the neuron, to normalize the analysis regarding the length of the neuron (Figure 27).

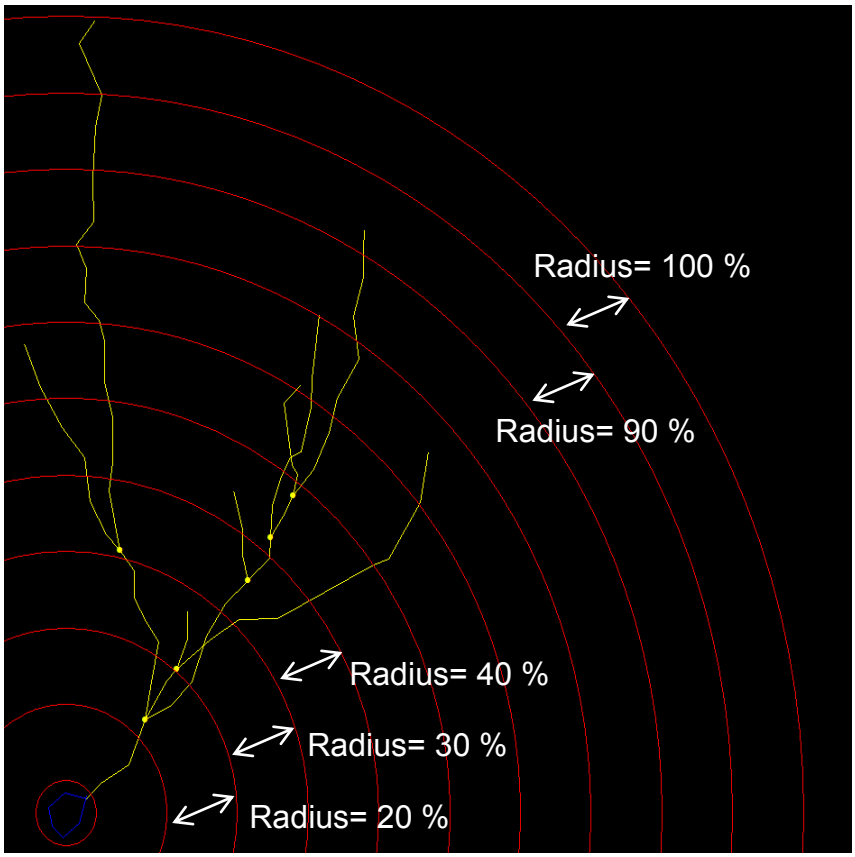


Figure 27: Sholl analysis. The dendritic arborization of adult-born neurons was measured using Sholl analysis. The arborization of newborn neurons was divided in radiuses, and the total dendritic length was calculated between each radius. The space between each radius was defined as 10% of the maximal length of the neuron, in order to normalize the data regarding the size of the neurons.

Maximal dendritic extension and migration were measured on maximal intensity projections. Maximal dendritic extension was defined as the ratio between the length from the cell body of the neuron to the tip of its longest dendrite and the distance from the cell body to the end of the molecular layer of the dentate gyrus (Figure 28) [108]. Neuronal migration was measured as the ratio between the length from the beginning of the granule cell layer and the beginning of the cell body of the neuron and the thickness of the granule cell layer (Figure 28).

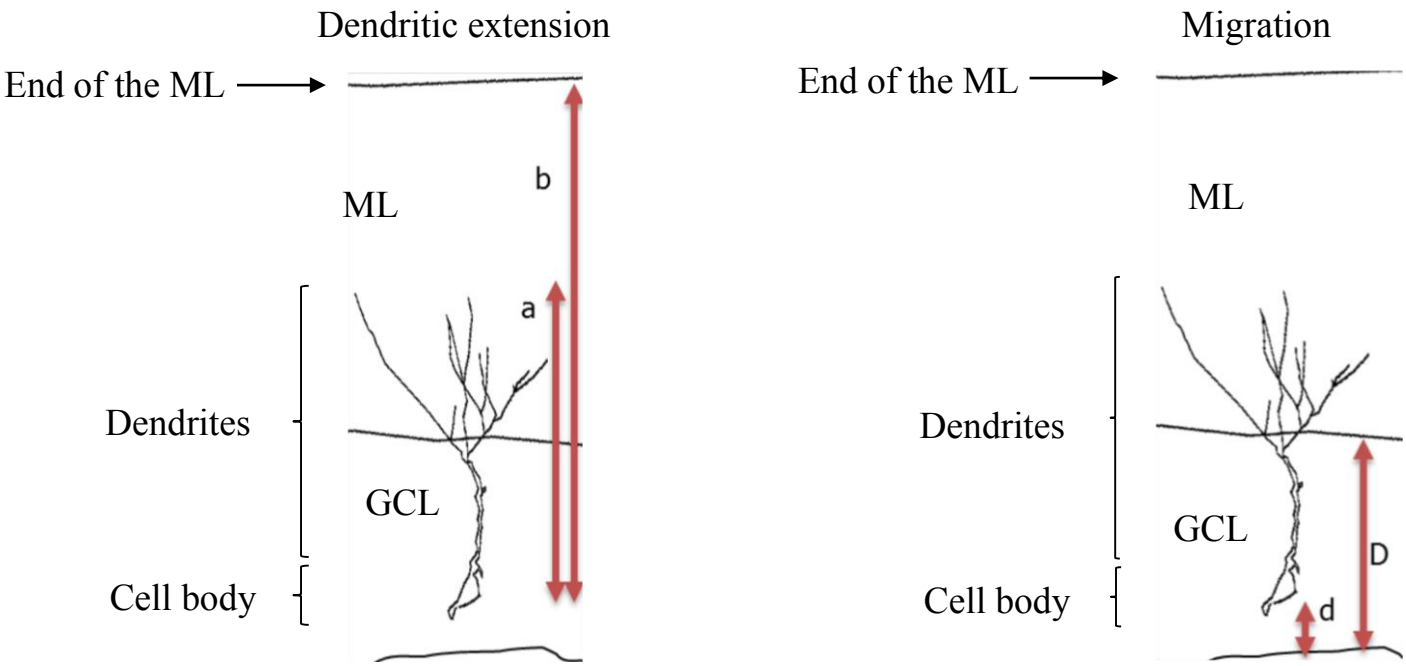


Figure 28: Measurements of the maximal dendritic extension and migration of newborn neurons. The maximal dendritic extension was defined as a/b*100. The migration was calculated as d/D. GCL: Granule cell layer; ML: Molecular layer

Adult-born neuron survival was assessed by injecting 8 mice with the same mixture of retroviruses, and sacrifying 4 mice at 14 dpi and 4 mice at 28 dpi. For each mouse, the total number of overexpressing and control neurons were counted, and the ratio between the total number of overexpressing neurons and the total number of control neurons was calculated at the two timepoints. If overexpressing adult-born neurons displayed increased survival compared to controls, the ratio was expected to increase between 14 and 28 dpi. Inversely, if overexpressing neurons survived less than controls, the ratio would decrease between these two timepoints. Finally, if survival was not influenced by the gene overexpression, the ratio would not change (Figure 29).

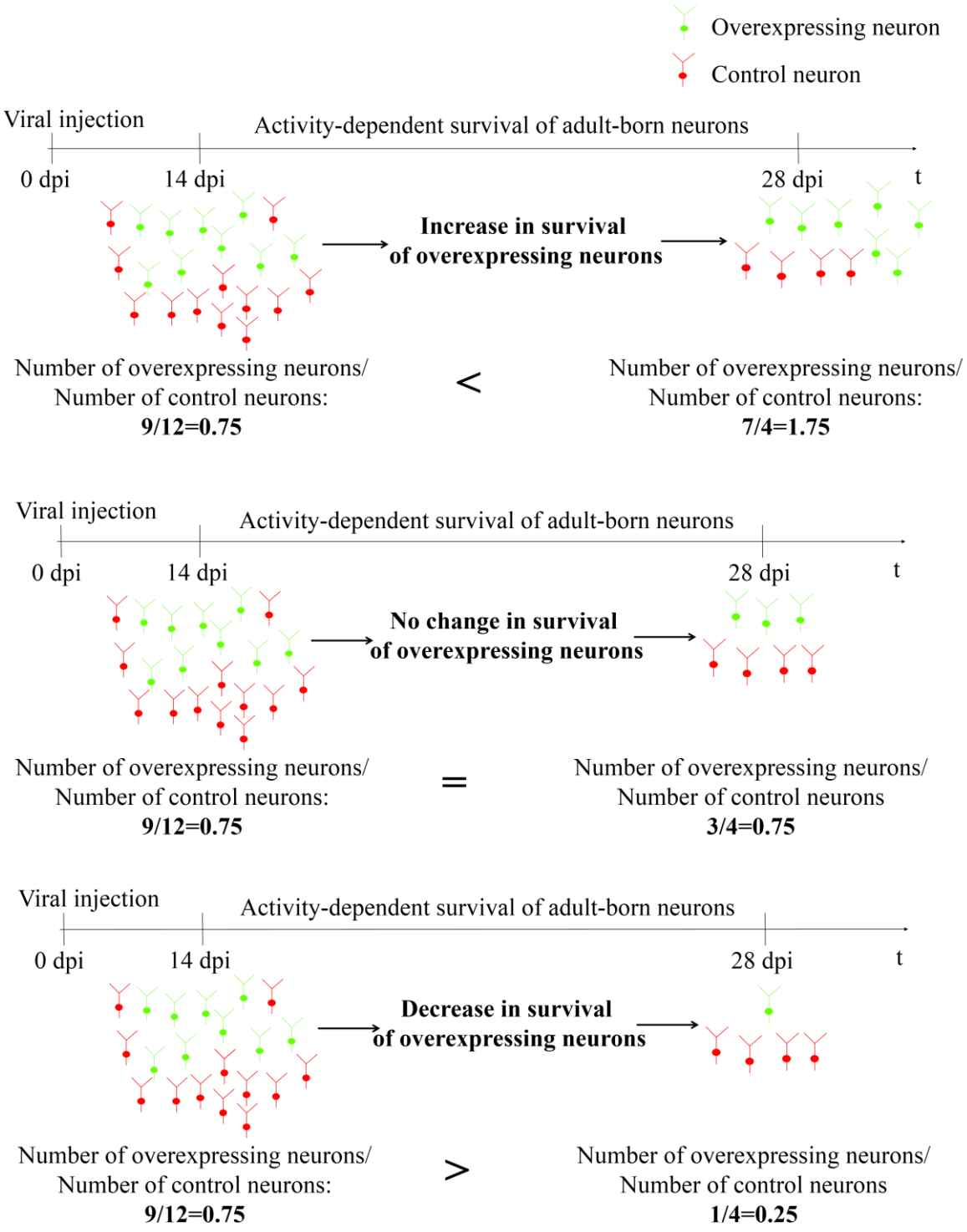


Figure 29: Assessment of the differences of survival between control and overexpressing neurons between 14 and 28 dpi. All mice were injected with the same mixture of retroviruses at Day 0 (0 dpi). Half of the mice were sacrified at 14 dpi, and the other half was sacrified at 28 dpi. For each mouse, the total number of overexpressing and control neurons were counted, and the ratio between the total number of overexpressing neurons and the total number of control neurons was calculated. If the survival of overexpressing adult-born neurons was increased, the ratio was expected to increase between 14 and 28 dpi (upper panel). If survival did not change, the ratio was expected to remain

constant between the two timepoints (middle panel). Finally, if survival was decreased, the ratio was expected to decrease between the two timepoints (lower panel).

Statistical analyses

Hypothesis testing was two-tailed. All analyses were performed using GraphPad Prism 6 (Graphpad Software, Inc., La Jolla, California, USA). First, Shapiro-Wilk tests were performed on each group of data to test for distribution normality. When the distribution was not normal, the non-parametric Mann-Whitney test was applied. When the distribution was normal, the equality of variances of the groups was tested and the adequate unpaired t-test was used. Data are presented as mean ± SEM. For Sholl analyses, the total dendritic length of control and overexpressing neurons was calculated and used for statistical analyses.

Results

To overexpress adhesion molecules in mouse adult-born hippocampal neurons, we injected the dentate gyrus of adult mice with a mixture of two retrovirus, one carrying a fluorescent protein reporter expression cassette (cag-XFP, control cassette), and the other one carrying the expression cassette of the gene of interest and another fluorescent protein reporter (cag-XFP-2A-gene of interest, cassette of overexpression). Mice were sacrified at different timepoints after injection, and the migration, maturation, integration and survival of adult-born neurons were evaluated using confocal microscopy (Figure 26).

dnSynCAM1 expression decreases glutamatergic synapse and mossy fiber terminal maturation, and alters the survival of newborn neurons

To investigate whether SynCAM1 was involved in the regulation of synaptogenesis on adult-born neurons, we first expressed the dominant negative form of SynCAM1 (dnSynCAM1) in adult-born neurons, and we studied their synaptic integration and survival. As SynCAM1 constitutive knock-out decreases dendritic spine density in hippocampal CA1 neurons [97], we first measured the dendritic spine density of adult-born neurons.

The proximal and second thirds of the molecular layer are innervated differently: the proximal third receives hilar and commissural afferences, whereas the second third receives input from the entorhinal cortex. Therefore, the proximal and second third of the molecular layer were analyzed separately. Surprisingly, at 21 and 28 days post-injection (dpi), dnSynCAM1 expression did not affect dendritic spine density (Figure 30). As dendritic spines usually receive

glutamatergic input, the density of dendritic spines reflects glutamatergic synapse density. Thus, dnSynCAM1 expression had no effect on glutamatergic synapse formation on adult-born neurons.

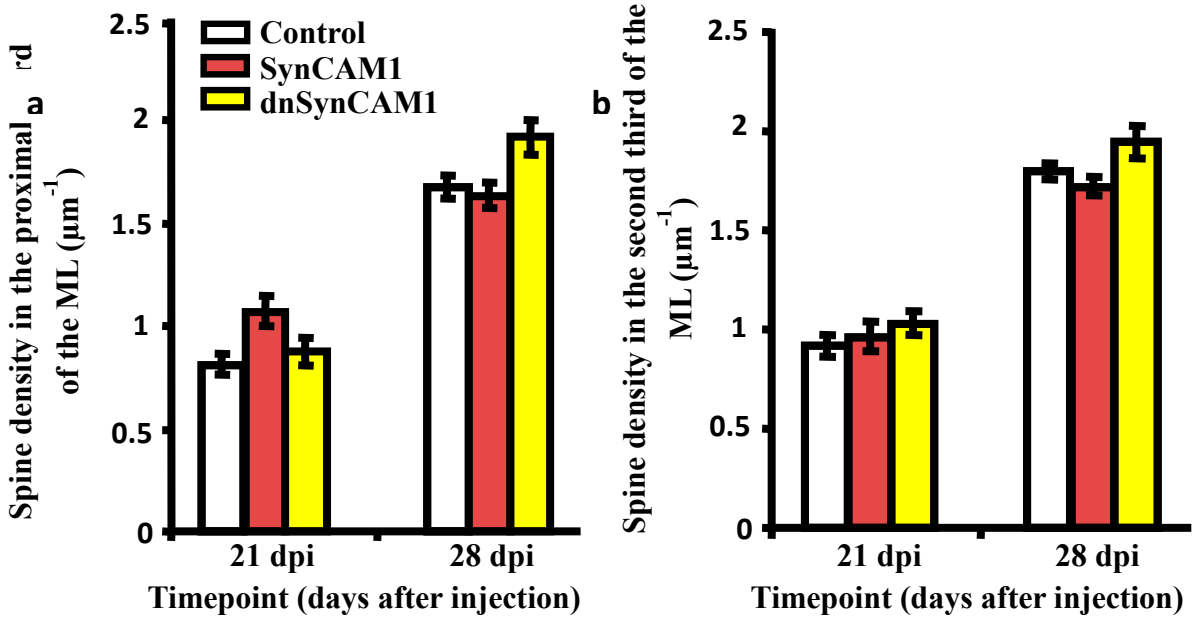


Figure 30: Effect of dnSynCAM1 expression or SynCAM1 overexpression on the dendritic spine density of adult-born hippocampal neurons. a, Dendritic spine density of newborn neurons in the proximal third of the molecular layer. b, Dendritic spine density of newborn neurons in the middle third of the molecular layer. Neither dnSynCAM1 expression nor SynCAM1 overexpression affected dendritic spine density in adult-born hippocampal neurons at 21 and 28 dpi. $n_{animals}$=4, $n_{neurons}$=40 per group

Sara et al. showed that SynCAM1 increased synapse efficacy in cultured hippocampal neurons [98]. Furthermore, synapse efficacy increases during synapse maturation, and dendritic spine morphology is related to excitatory synapse maturation in adult-born neurons: the proportion of mushroom spines increases with the age of the newborn neurons, whereas the proportion of filopodia decreases [19]. Thus, we then analyzed dendritic spine morphology of adult-born neurons (Figure 31). We classified the dendritic spines according to their maximal diameter into three categories: filopodia: diameter strictly inferior to 0.30 µm; thin spine: diameter comprised between 0.30 µm and 0.55 µm; mushroom spine : diameter strictly superior to 0.55 µm. At 28 dpi, dnSynCAM1 overexpression decreased the proportion of mushroom spines and increased the proportion of filopodia. No effect could be detected at 21 dpi. Our data suggest that dnSynCAM1 expression decreased excitatory synapse maturation at 28 dpi.

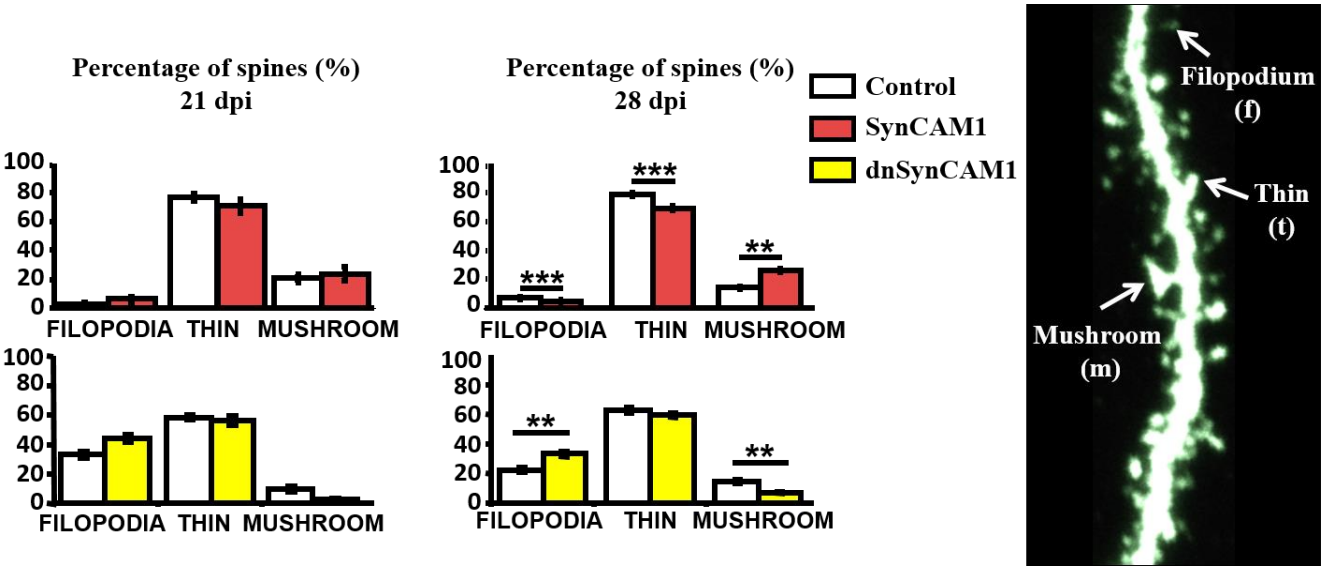


Figure 31: Effects of SynCAM1 expression or dnSynCAM1 expression on dendritic spine morphology at 28 dpi. Dendritic spines were classified into three categories according to their diameter: filopodia, thin, and mushroom spines. An example of each category is represented on the picture on the right. a, Percentage of filopodia, thin and mushroom spines of control and SynCAM1-overexpressing (upper panel) or dnSynCAM1-expressing neurons (lower panel) at 21 dpi. b, Percentage of filopodia, thin and mushroom spines of control and SynCAM1-overexpressing (upper panel) or dnSynCAM1-expressing neurons (lower panel) at 28 dpi. dnSynCAM1 expression increased the proportion of filopodia and decreased the proportion of mushroom spines, whereas SynCAM1 overexpression decreased the proportion of filopodia and thin spines and increased the proportion of mushroom spines in adult-born hippocampal neurons. $n_{animals}$=4, $n_{neurons}$=40 per group. **: p<0.01; ***: p<0.0001

As dendritic spine maturation was altered by dnSynCAM1 expression, and as SynCAM1 is located pre- and post-synaptically, we hypothesized that mossy fiber terminal maturation may also be affected by dnSynCAM1 expression in newborn granule cells. We studied different parameters of maturation of the axon terminals of adult-born neurons, i.e. the mossy fiber terminals (Figure 32). During mossy fiber terminal maturation, their area, perimeter and the number of extensions increase, whereas their circularity decreases. At 28 dpi, dnSynCAM1 expression decreased mossy fiber terminal area, perimeter, and number of extensions, and increased circularity.

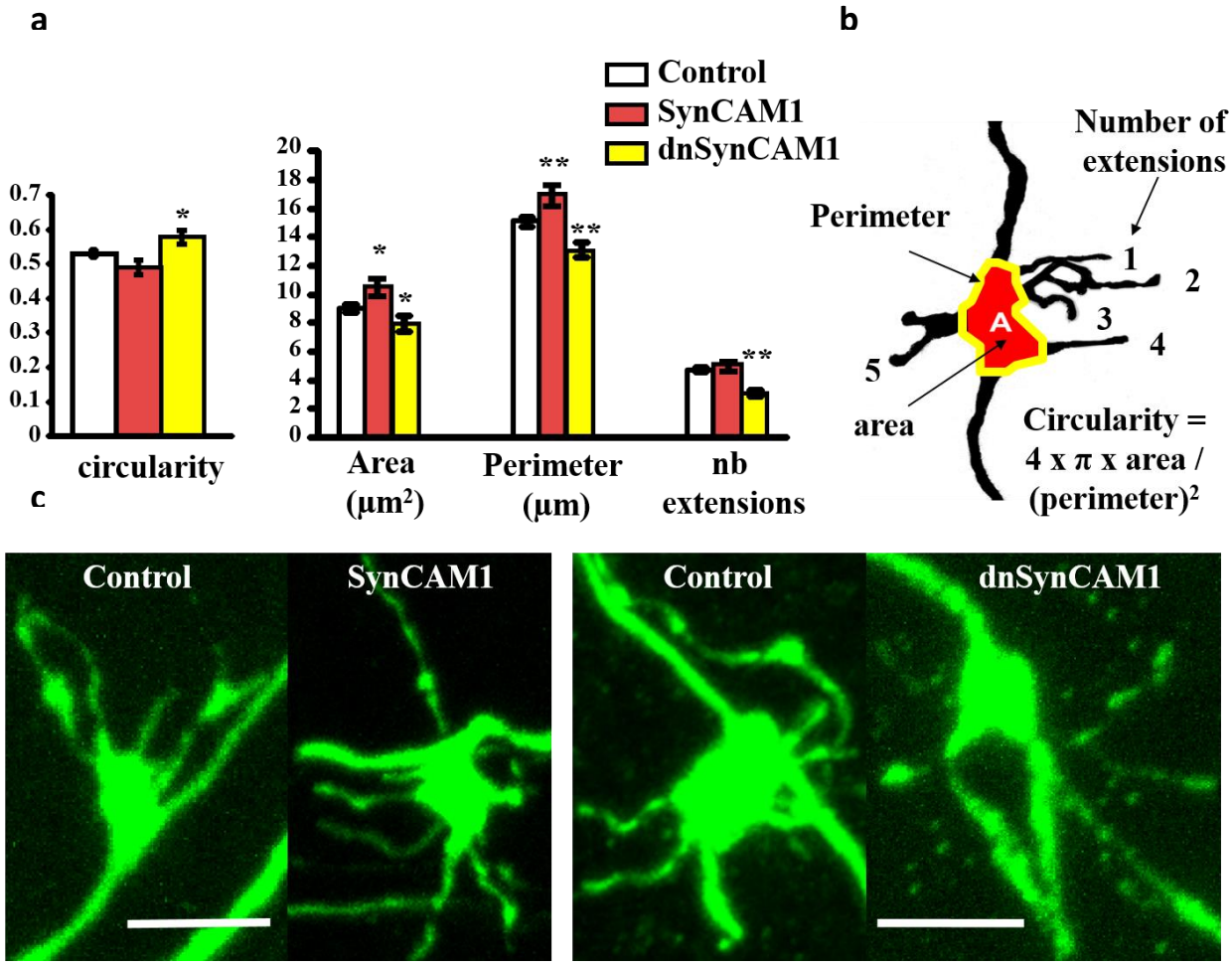


Figure 32: Effects of SynCAM1 expression or dnSynCAM1 expression on mossy fiber terminal (MFT) maturation at 28 dpi. a, Circularity, area, perimeter and number of extensions of MFT of control, SynCAM1-overexpressing and dnSynCAM1-expressing neurons. dnSynCAM1 expression decreased the area, perimeter, number of extensions, and increased the circularity of MFTs, whereas SynCAM1 overexpression increased the area and perimeter of MFTs. b, Measurement of the different parameters of MFT maturation. c, Representative confocal images of control and SynCAM1-overexpressing (left panel) or dnSynCAM1-expressing MFTs (right panel) at 28 dpi. Scale bars represent 5 µm. $n_{animals}$=4, n_{MFT}=40 to 60 per group. *: p<0.05; **: p<0.01

dnSynCAM1 expression also decreased the average axonal length of adult-born neurons: the axons of neurons expressing dnSynCAM1 reached less often the end of the CA3 region than the controls (Figure 33). Thus, dnSynCAM1 expression decreased mossy fiber terminal maturation and axon elongation in adult-born neurons.

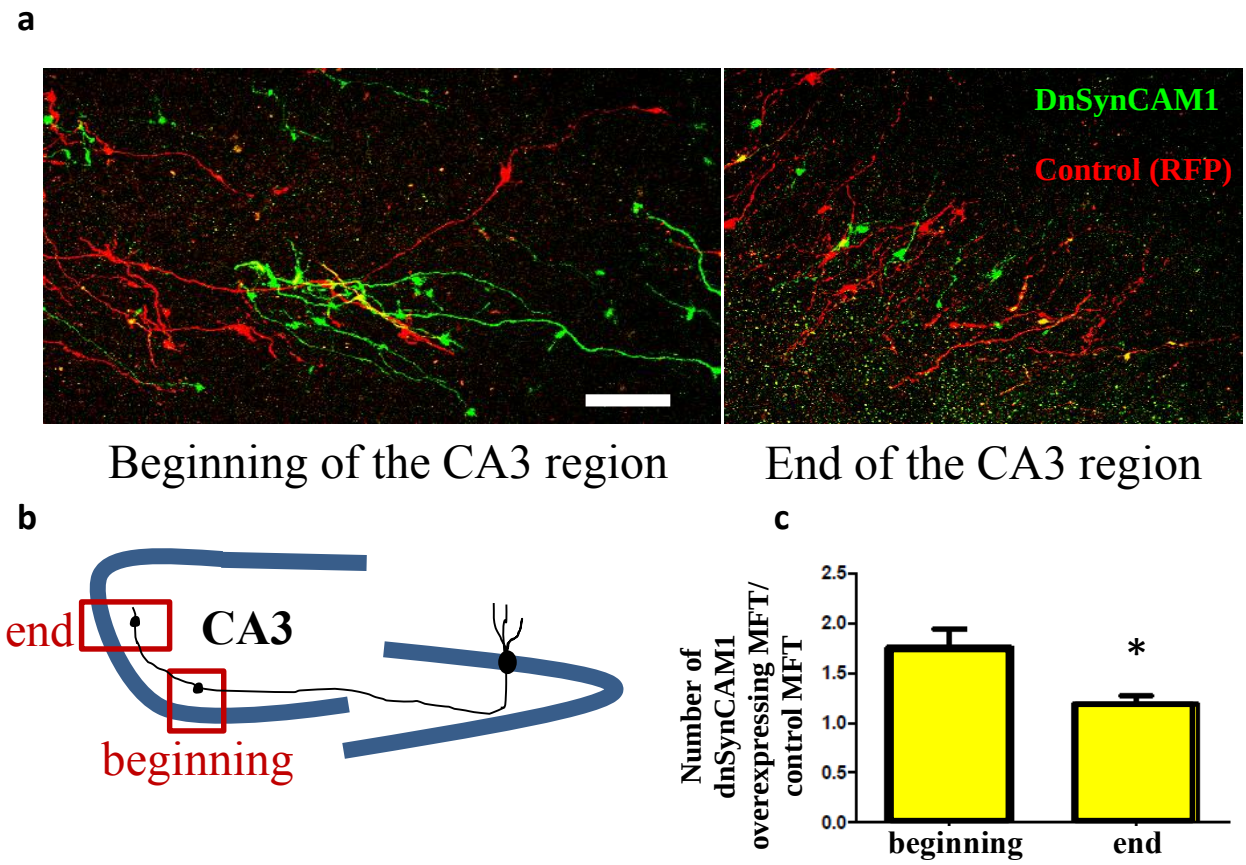


Figure 33: dnSynCAM1 expression decreases axonal length at 28 dpi. a, Representative maximal intensity projections of confocal microscope fields at the beginning and the end of the CA3 region. GFP-expressing MFTs are overexpressing dnSynCAM1, whereas RFP-expressing MFTs are control MFTs. b, Localization of the fields at the beginning and the end of the CA3 region. c, The ratio between the number of dnSynCAM1-overexpressing MFTs and the number control MFTs decreased between the beginning and the end of the CA3 region. The scale bar represents 20 µm. $n_{animals}$=4. *: p<0.05

The maturation of the dendritic arborization and synaptogenesis may be linked: Ge and colleagues showed that knock-down of NKCC1 led to marked defects in both synapse formation and dendritic development of newborn neurons [36]. Sholl analysis showed a transient increase in dendritic arborization by dnSynCAM1 expression at 21 dpi (Figure 34).

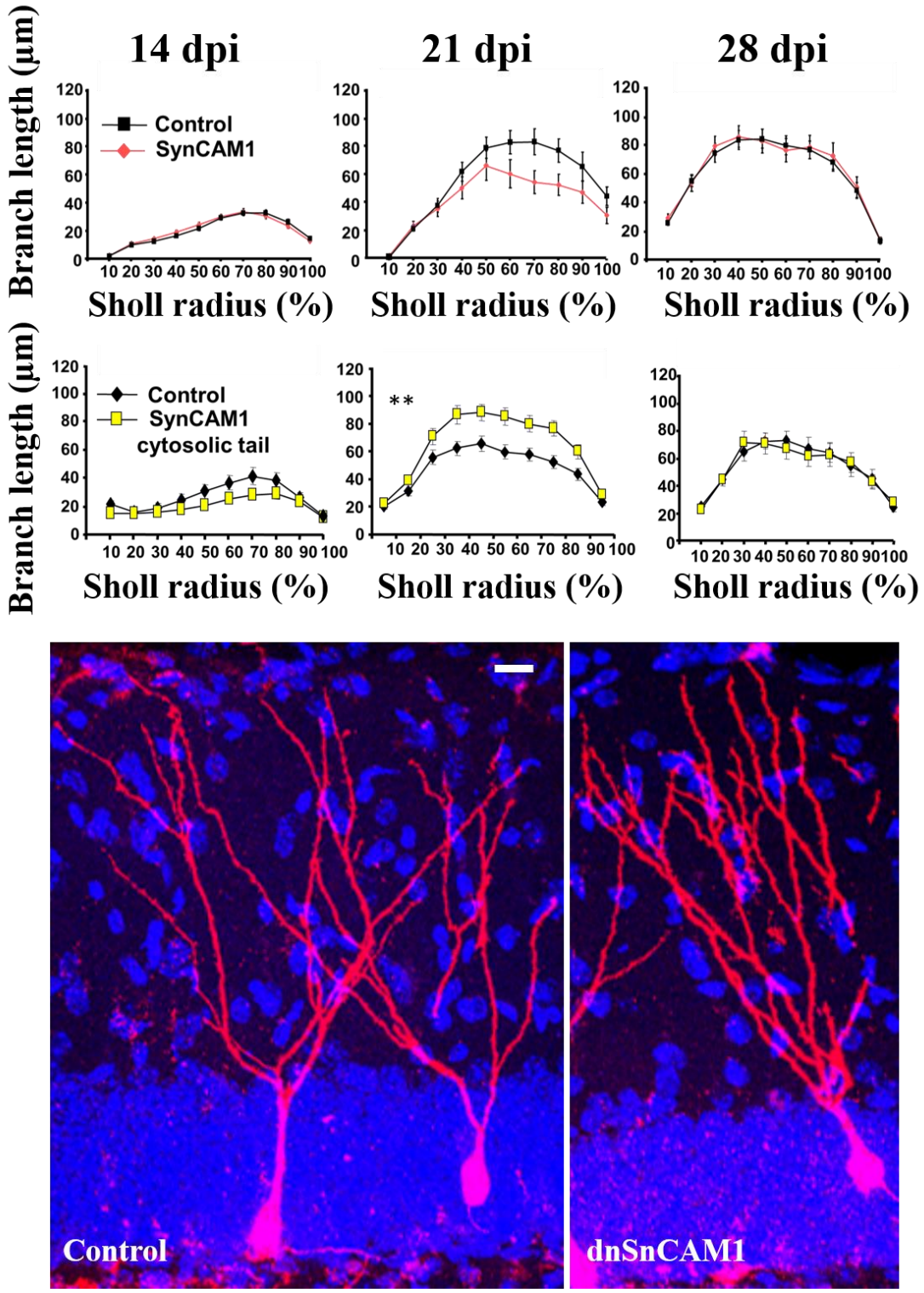


Figure 34: Sholl analysis of dnSynCAM1-expressing and SynCAM1-overexpressing neurons. From left to right: Dendritic branch length of control and SynCAM1-overexpressing (upper panel) or dnSynCAM1-expressing (lower panel) adult-born neurons between each Sholl radius, at 14, 21 and 28 dpi. Sholl radiuses are expressed as a percentage of the total length of the neuron. Lower panel:

representative confocal images of control (left) and dnSynCAM1-expressing (right) neurons at 21 dpi. Scale bar represents 10 µm. $n_{animals}$=4, $n_{neurons}$=40 per group. **: p<0.01

However, these differences were normalized at 28 dpi, indicating that expression of dnSynCAM1 had no long-lasting effects on the development of the dendritic arborization of adult-born neurons. Dendritic extension was not affected by dnSynCAM1 expression (Figure 35).

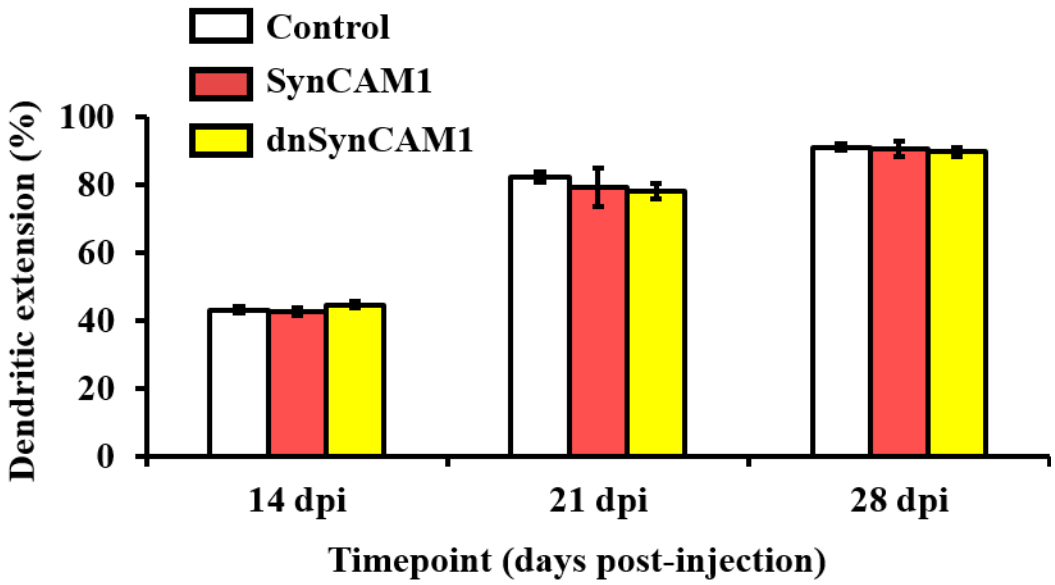


Figure 35: Maximal dendritic extension of newborn hippocampal neurons is neither affected by SynCAM1 overexpression nor by dnSynCAM1 expression. Maximal dendritic extension of control, SynCAM1-overexpressing and dnSynCAM1-expressing neurons at 14, 21 and 28 dpi. No significant difference was measured between the maximal dendritic extension of control and SynCAM1-overexpressing or dnSynCAM1-expressing neurons. $n_{animals}$=4, $n_{neurons}$=40 per group

dnSynCAM1 expression had no effect on the migration of newborn neurons (Figure 36).

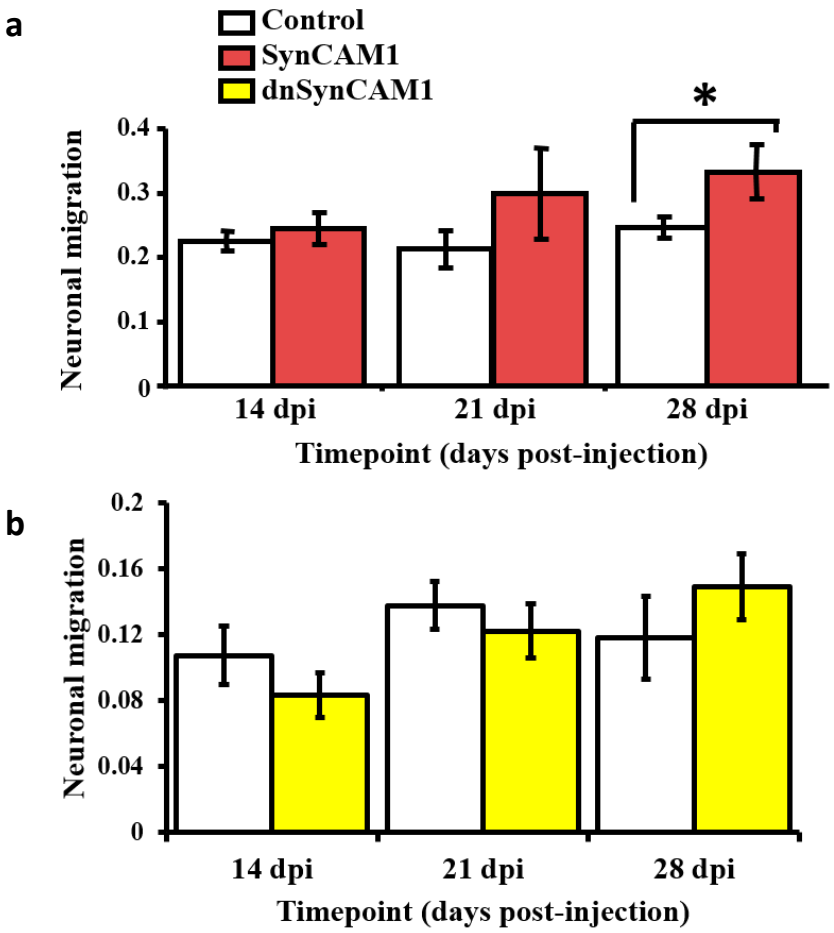
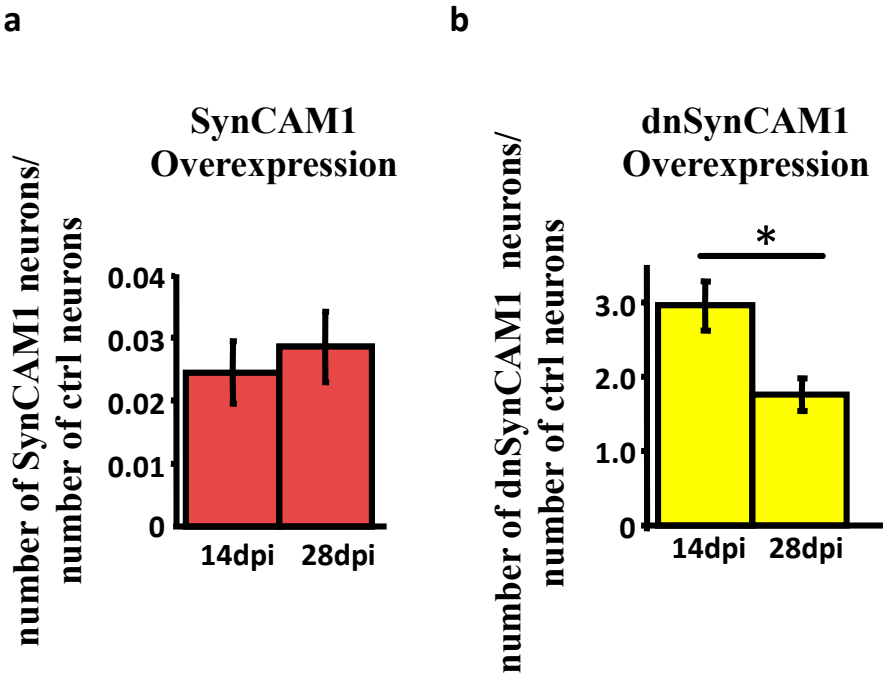


Figure 36: dnSynCAM1 expression did not affect newborn neuron migration, whereas SynCAM1 overexpression slightly increased neuronal migration at 28 dpi. Migration of control and SynCAM1-overexpressing (a) or dnSynCAM1-expressing (b) neurons in the GCL at 14, 21 and 28 dpi. SynCAM1 overexpression increased newborn neuron maturation at 28 dpi. $n_{animals}$=4, $n_{neurons}$=40 per group. *: $p<0.05$

As dnSynCAM1 decreased both glutamatergic synapse and MFT maturation, we next investigated changes in survival provoked by the expression of dnSynCAM1 in newborn neurons. Adult-born granule cell death occurs predominantly before 4 weeks of maturation. We evaluated adult-born neuron survival between 14 and 28 dpi. We injected mice with the same mixture of viruses, and sacrified half of them at 14 dpi, and the other half at 28 dpi. We then measured the ratio between the number of overexpressing neurons and the number of control neurons for each mouse, at both timepoints. If overexpressing adult-born neurons have increased survival compared to control, the ratio is expected to increase between 14 and 28 dpi. Inversely, if overexpressing neurons survive less than controls, the ratio will decrease between these two timepoints. Finally, if survival is not influenced by the gene overexpression, the ratio will not change (Figure 29).

The average ratio between dnSynCAM1-expressing and control neurons decreased between 14 and 28 dpi. Thus, dnSynCAM1 expression decreased adult-born neuron survival (Figure 37). These results suggest that glutamatergic synapse maturation is an important factor for adult-born neuron survival.

Figure 37: dnSynCAM1 expression decreases the survival of hippocampal adult-born neurons



between 14 and 28 dpi, whereas SynCAM1 overexpression has no effect. a, Ratios between the total number of SynCAM1-overexpressing neurons and the number of control neurons at 14 and 28 dpi. b, Ratios between the total number of dnSynCAM1-expressing neurons and the number of control neurons at 14 and 28 dpi. $n_{animals}$=4 per group. *: $p<0.05$

SynCAM1 overexpression increases glutamatergic synapse and mossy fiber terminal maturation without increasing the survival of hippocampal adult-born neurons

As overexpression of dnSynCAM1 in adult-born neurons decreased glutamatergic synapse and MFT maturation and had a deleterious outcome of their survival, we hypothetized that SynCAM1 overexpression may increase glutamatergic synapse and MFT maturation in adult-born neurons and improve their survival. Thus, we overexpressed the SynCAM1 in adult-born neurons, and we characterized changes their synaptic integration and survival with the same parameters we used for dnSynCAM1-expressing neurons.

At 28 dpi, SynCAM1 overexpression increased the proportion of mushroom spines, while decreasing the proportion of thin spines and filopodia (Figure 31). No effect could be detected at 21 dpi. SynCAM1 overexpression also increased MFT area and perimeter at 28 dpi (Figure 32). Thus, as expected, SynCAM1 overexpression had the opposite effect as dnSynCAM1 expression, and increased excitatory synapse and MFT maturation at 28 dpi.

At 21 and 28 dpi, SynCAM1 overexpression did not affect dendritic spine density (Figure 30). Thus, neither dnSynCAM1 expression nor SynCAM1 overexpression had an effect on glutamatergic synapse formation on adult-born neurons. Similarly, Sholl analysis and analysis of dendritic extension showed no effect of SynCAM1 overexpression on the dendritic arborization and dendritic extension of adult-born neurons (Figures 34-35). However, the migration of SynCAM1-overexpressing neurons was increased at 28 dpi, although the neurons remained in the GCL (Figure 36).

Surprisingly, the ratio between SynCAM1-overexpressing neurons and control neurons was unchanged between 14 and 28 dpi, indicating that SynCAM1 overexpression did not modify adult-born neuron survival (Figure 37). Together, these results suggest that although glutamatergic synapse maturation is an important factor for adult-born neuron survival, increasing glutamatergic synapse maturation may not be sufficient to increase newborn neuron survival.

Neuroligin-1B overexpression increases glutamatergic synaptogenesis without affecting newborn neuron survival

We next investigated whether acting on synapse formation per se would modify neuronal survival. NL1B overexpression increases glutamatergic synaptogenesis in cultured hippocampal neurons, whereas it does not affect GABAergic synaptogenesis [106]. We first assessed the effect of NL1B overexpression on the synaptogenesis of adult-born neurons in vivo. As expected, NL1B overexpression increased dendritic spine density in the second third of the molecular layer in adult-born neurons at 21 and 28 dpi, whereas we could not detect any changes in the proximal third of the molecular layer (Figure 38).

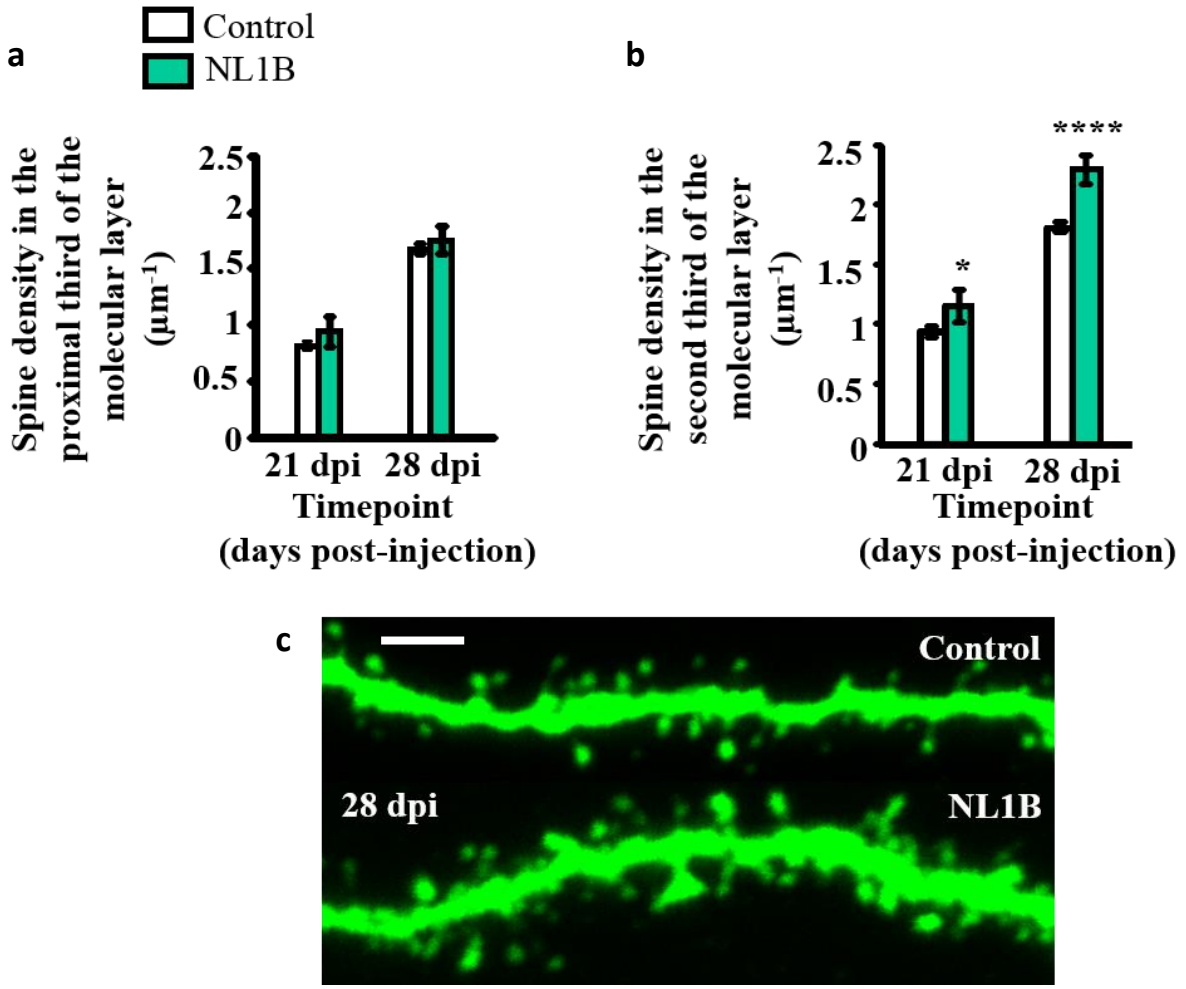


Figure 38: NL1B overexpression increased dendritic spine density of newborn neurons in the second third of the molecular layer. a, Spine density of NL1B-overexpressing and control neurons in the proximal third of the molecular layer. b, Spine density of NL1B-overexpressing and control neurons in the middle third of the molecular layer. c, Representative confocal maximal intensity projections of control and NL1B-overexpressing neurons at 28 dpi. The scale bar represents 5 µm. $n_{animals}$=4, $n_{neurons}$=40 per group. *: p<0.05; ****: p<0.00001

In contrast with SynCAM1 overexpression, NL1B overexpression did not affect dendritic spine diameter (Figure 39).

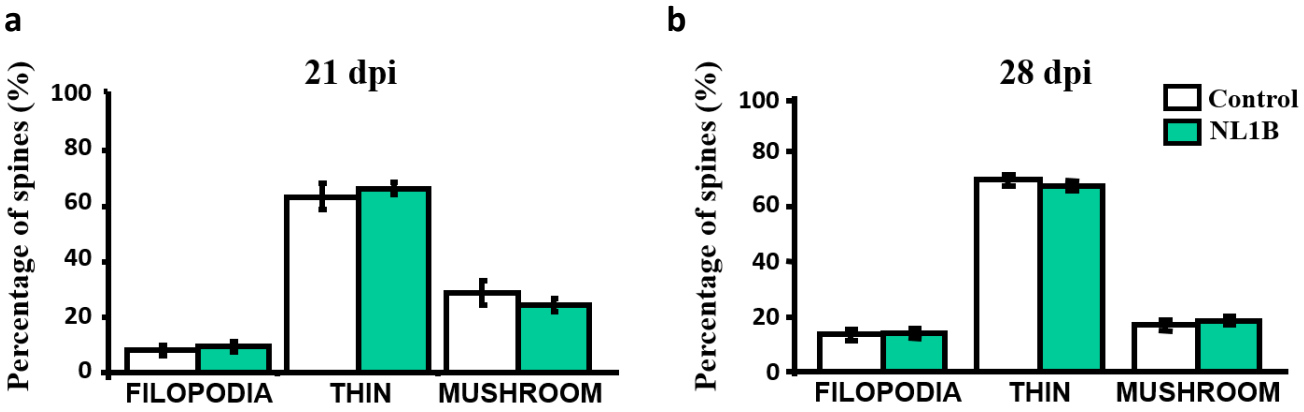


Figure 39: NL1B overexpression did not affect the dendritic spine morphology of newborn neurons. Percentage of filopodia, thin and mushroom spines in control and NL1B-overexpressing neurons at 21 (a) and 28 (b) dpi. $n_{animals}$=4, $n_{neurons}$=40 per group

Also, NL1B overexpression did not affect mossy fiber terminal area, perimeter, circularity, or number of extensions (Figure 40).

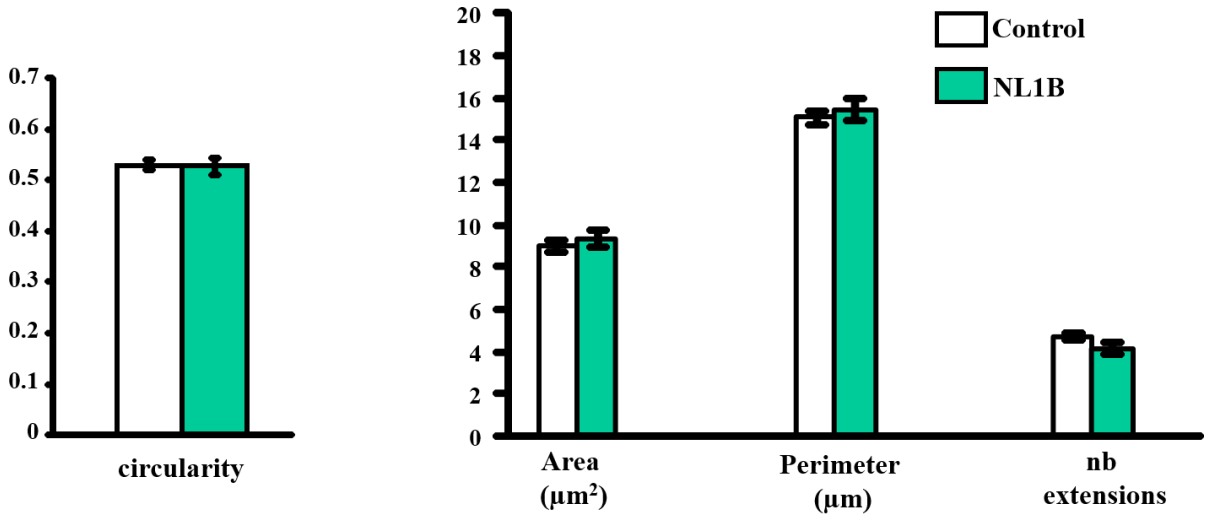


Figure 40: NL1B overexpression did not affect the MFT maturation of newborn neurons. Circularity, area, perimeter and number of extensions of MFT of control and NL1B-overexpressing neurons. $n_{animals}$=4, n_{MFT}=40 to 60 per group

We next evaluated whether NL1B overexpression induced changes in the maturation of the dendritic arborization of newborn hippocampal neurons. Sholl analysis showed no effect of NL1B overexpression on dendritic arborization (Figure 41).

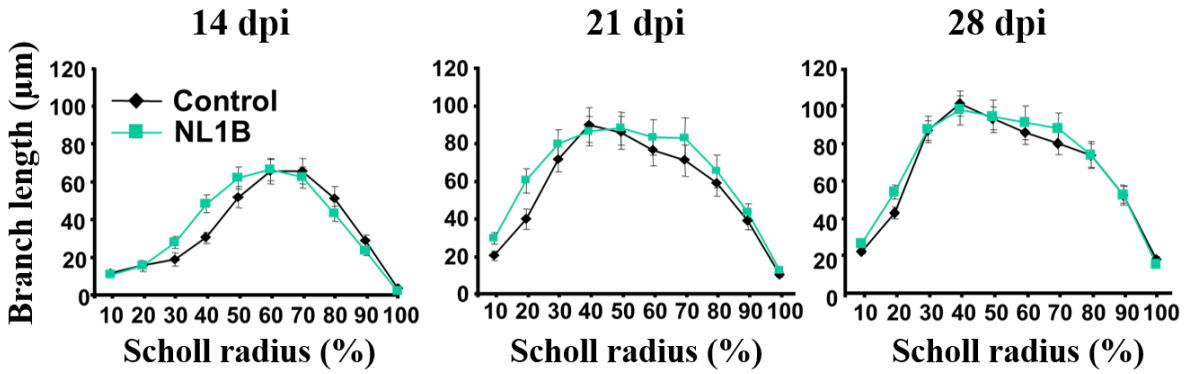


Figure 41: NL1B overexpression did not affect the development of the dendritic arborization of newborn neurons. From left to right: Dendritic branch length of control and NL1B-overexpressing adult-born neurons between each Sholl radius, at 14, 21 and 28 dpi. Sholl radiuses are expressed as a percentage of the total length of the neuron. $n_{animals}$=4, $n_{neurons}$=40 per group

Furthermore, the dendritic extension and migration of newborn neurons were not affected by NL1B overexpression (Figures 42).

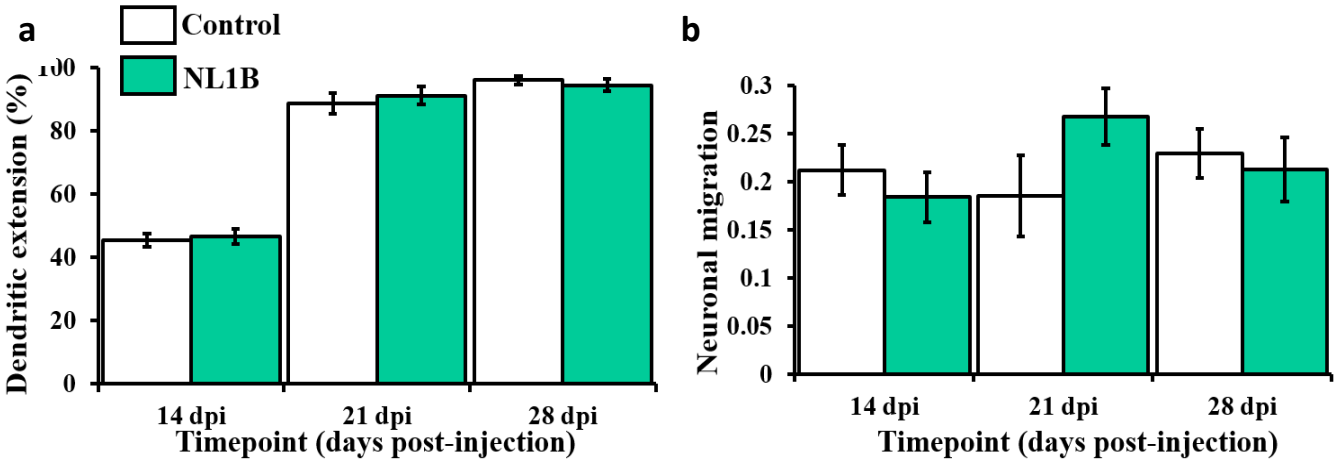


Figure 42: NL1B overexpression neither affected the maximal dendritic extension nor the migration of newborn neurons. a, Maximal dendritic extension of control and NL1B-overexpressing neurons at 14, 21 and 28 dpi. b, Migration in the GCL of control and NL1B-overexpressing neurons at 14, 21 and 28 dpi. $n_{animals}$=4, $n_{neurons}$=40 per group

Finally, we measured the survival of NL1B-overexpressing adult-born neurons between 14 and 28 dpi. The survival of adult-born neurons was not significantly increased by NL1B overexpression (Figure 43).

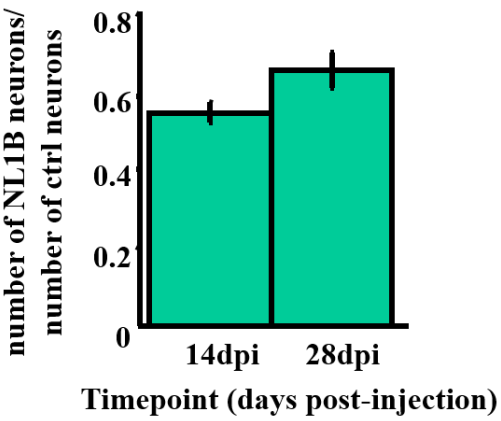


Figure 43: NL1B overexpression did not affect newborn neuron survival between 14 and 28 dpi. Ratios between the total number of NL1B-overexpressing neurons and the number of control neurons at 14 and 28 dpi. $n_{animals}$=4

Together, these results suggest that the increase in glutamatergic synapse density produced by NL1B overexpression is not sufficient to increase adult-born neuron survival, or that increasing glutamatergic synapse density in newborn neurons is not sufficient to improve their survival.

Neuroligin-2A overexpression increases glutamatergic and GABAergic synaptogenesis, and increases newborn neuron survival

We next investigated whether increasing both glutamatergic and GABAergic synaptogenesis would increase neuronal survival. We overexpressed NL2A in adult-born neurons, and we studied their maturation, integration and survival.

We first measured the effect of NL2A overexpression on the formation of glutamatergic and GABAergic synapses by adult-born neurons (Figure 44). NL2A overexpression increased dendritic spine density in the proximal and second thirds of the molecular layer at 21 dpi, whereas at 28 dpi dendritic spine density was only increased in the proximal third of the molecular layer. To measure GABAergic synapse density on adult-born neurons dendrites, brain slices were immunostained with antibodies directed against VGAT and $GABA_AR$, and we quantified the density of appositions of VGAT-positive and $GABA_AR$-positive puncta. GABAergic synapse density was increased in NL2A-overexpressing neurons at 21 dpi.

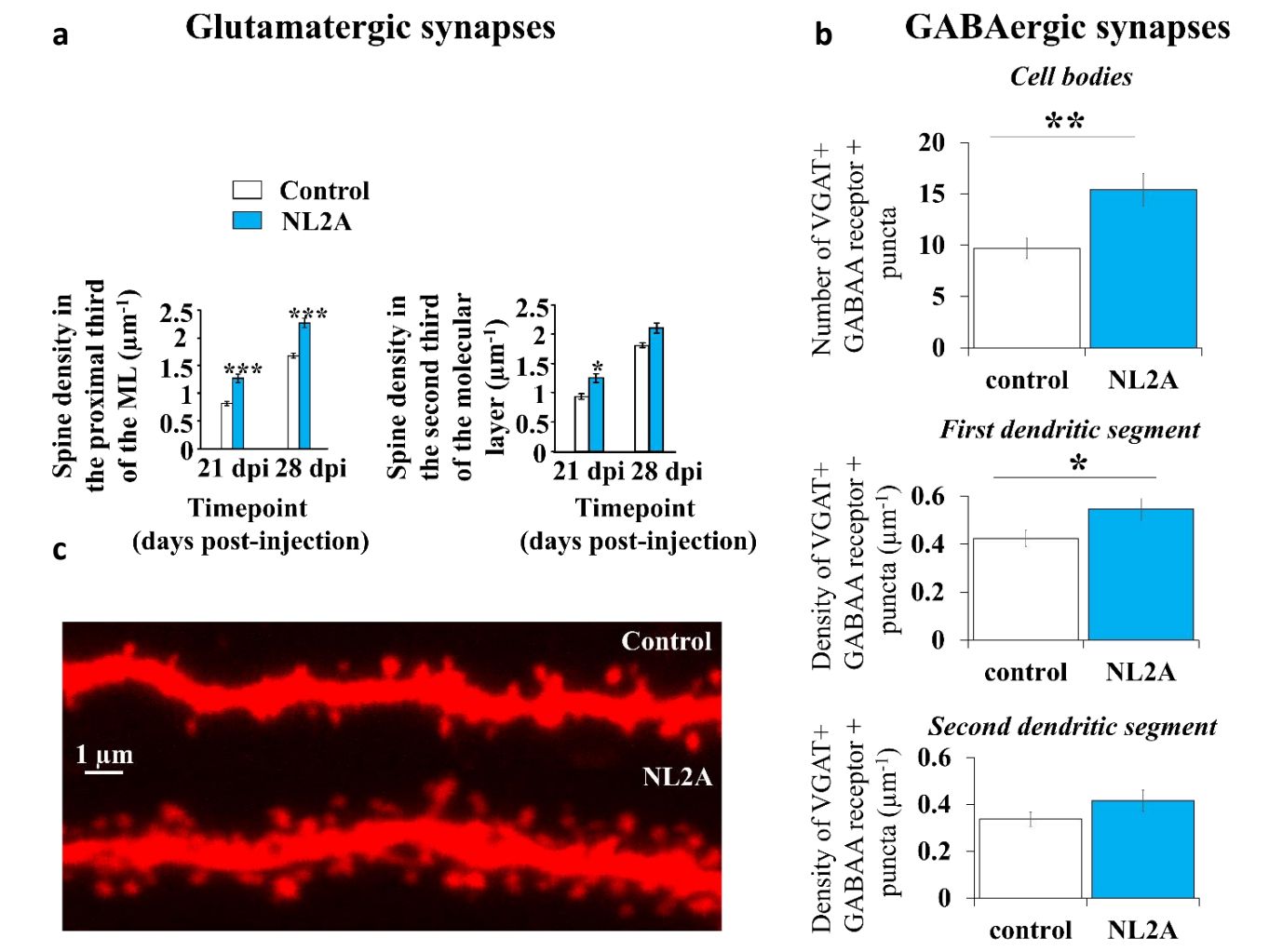


Figure 44: NL2A overexpression increases newborn neuron dendritic spine and GABAergic synapse density in adult-born neurons. a, Dendritic spine density in the proximal (left panel) and the middle (right panel) third of the molecular layer of control and NL2A-overexpressing neurons at 21 and 28 dpi. At 21 dpi, NL2A overexpression increased dendritic spine density in the first and second thirds of the ML. At 28 dpi, NL2A overexpression increased dendritic spine density in the first third of the ML. b, Density of GABAergic synapses in control and NL2A-overexpressing neurons at 21 dpi. At 21 dpi, NL2A overexpression increased the number of GABAergic synapses on the cell bodies of newborn neurons,

and the density of GABAergic synapses on the first dendritic segment of the neurons. The first dendritic segment of newborn neurons was defined at the segment that was adjacent to the cell body. The second dendritic segment was adjacent to the first one. c, Representative confocal maximal intensity projections of dendritic segments from control and NL2A-overexpressing neurons in the first third of the molecular layer at 28 dpi. d, Representative confocal plans of 21-day-old newborn neurons in slices immunostained for VGAT and $GABA_A$ receptor. Arrows show GABAergic synapses, defined as appositions of $GABA_AR$+ and VGAT+ puncta. From left to right: cell body, first dendritic segment, and second dendritic segment of a newborn neuron. $n_{animals}$=4, $n_{neurons}$=40 per group. *: p<0.05; **: p<0.01

We next assessed the effect of NL2A overexpression on dendritic spine and mossy fiber terminal maturation (Figure 45). NL2A overexpression only had a slight effect on dendritic spine morphology: at 28 dpi, the proportion of filopodia was increased, but the proportion of thin and mushroom spines remained unchanged. Similarly, NL2A overexpression slightly increased mossy fiber terminal area at 28 dpi, without changing the perimeter, number of extensions and circularity. This indicates that NL2A overexpression slightly increased glutamatergic synapse and mossy fiber terminal maturation.

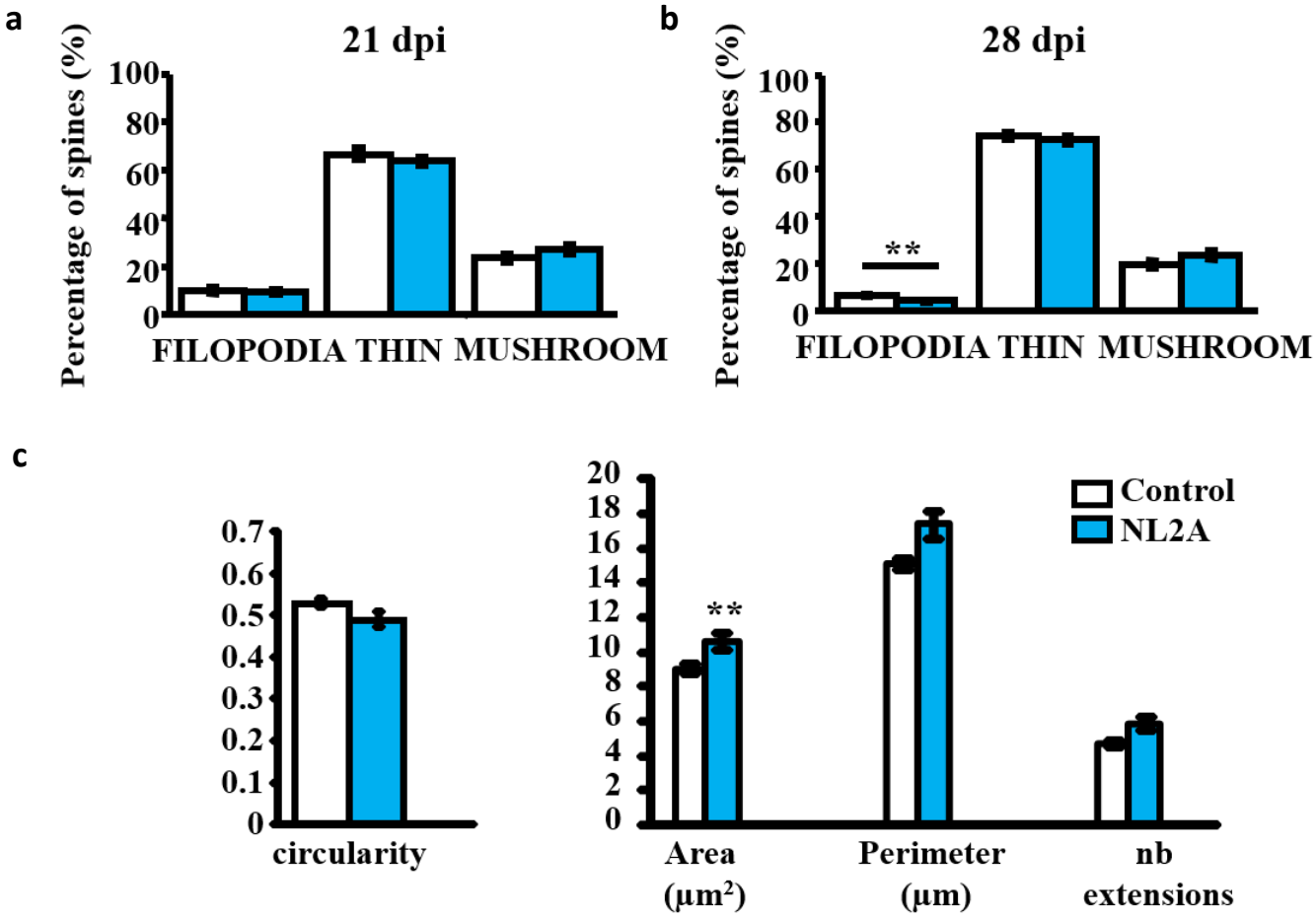


Figure 45: Effect of NL2A overexpression on dendritic spine morphology and mossy fiber terminal maturation. Percentage of filopodia, thin and mushroom spines in control and NL2A-overexpressing neurons at 21 (a) and 28 (b) dpi NL2A overexpression increased the proportion of filopodia in hippocampal newborn neurons at 28 dpi but not at 21 dpi. c, Circularity, area, perimeter and number of extensions of MFT of control and NL2A-overexpressing neurons. NL2A overexpression increased MFT area without changing the other morphological parameters at 28 dpi. $n_{animals}$=4, $n_{neurons}$=40 per group, n_{mft}=40 to 60 per group. **: p<0.01

Sholl analysis showed a transient increase in neuronal dendritic arborization at 14 dpi in NL2A-overexpressing neurons, which was normalized at 21 dpi (Figure 46).

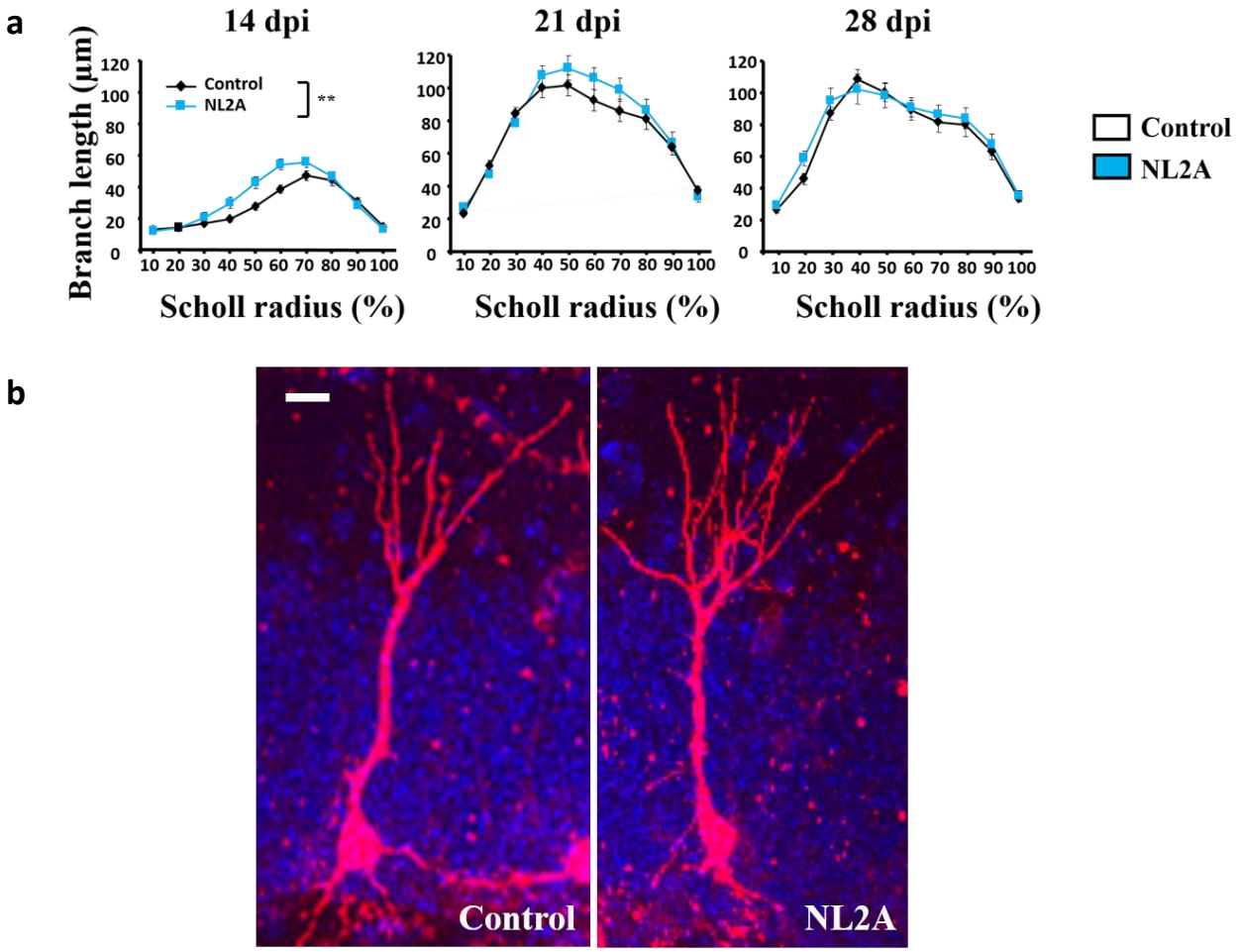


Figure 46: NL2A overexpression transiently increased newborn neuron dendritic arborization. a, From left to right: Dendritic branch length of control and NL2A-overexpressing adult-born neurons between each Sholl radius, at 14, 21 and 28 dpi. Sholl radiuses are expressed as a percentage of the total length of the neuron. NL2A overexpression increased neuronal dendritic arborization at 14 dpi, but the effect was normalized at 21 dpi. b, Representative confocal maximal intensity projections of control (left) and NL2A overexpressing (right) newborn hippocampal neurons. The scale bar represents 10 µm. $n_{animals}$=4, $n_{neurons}$=40 per group. **: p<0.01

Dendritic extension was not affected by NL2A overexpression, and the migration of NL2A-overexpressing neurons was only transiently increased at 21 dpi (Figure 47).

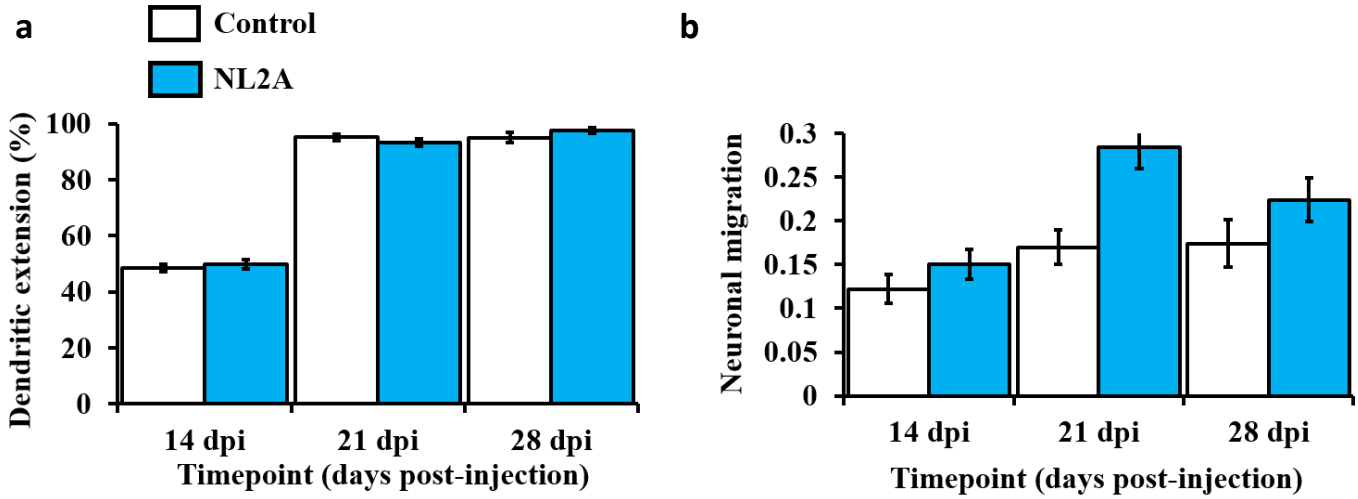


Figure 47: NL2A overexpression neither affected the dendritic extension nor the migration of newborn hippocampal neurons. a, Maximal dendritic extension of control and NL2A-overexpressing neurons at 14, 21 and 28 dpi. b, Migration in the GCL of control and NL2A-overexpressing neurons at 14, 21 and 28 dpi. $n_{animals}$=4, $n_{neurons}$=40 per group

Interestingly, NL2A overexpression increased adult-born neuron survival by two-fold (Figure 48).

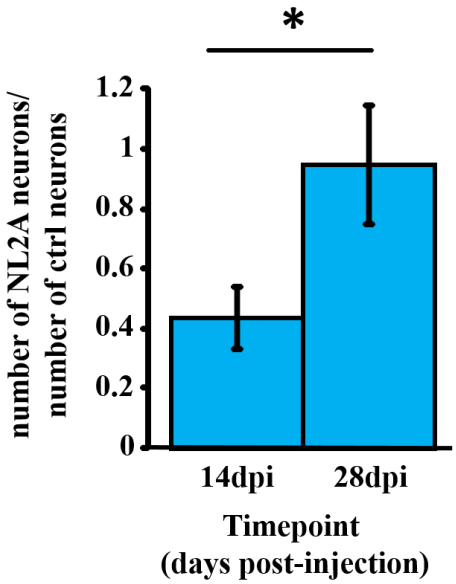


Figure 48: NL2A overexpression increased newborn neuron survival between 14 and 28 dpi. Ratios between the total number of NL2A-overexpressing neurons and the number of control neurons at 14 and 28 dpi. $n_{animals}$=4. *: $p<0.05$

Finally, at 14 dpi, the number of aberrant, i.e. neurons with a cell body located outside of the granule cell layer or displaying changes in their polarity, NL2A-overexpressing neurons and control neurons were comparable, suggesting that, if NL2A overexpression increases neuronal survival, it does not lead to the production of neurons that are aberrantly integrated into the network (Figure 49).

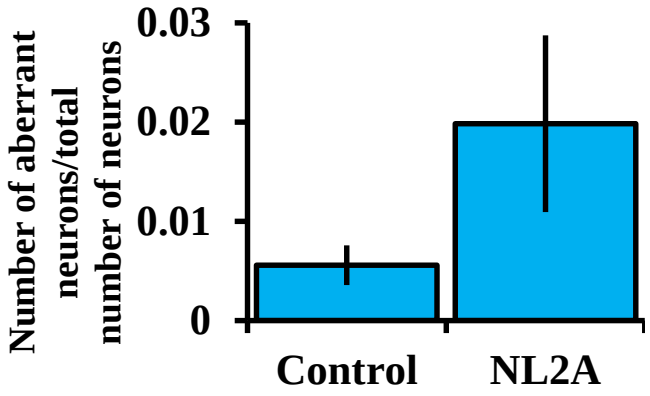


Figure 49: NL2A overexpression did not significantly increase the number of aberrant neurons at 14 dpi. Ratios between the number of aberrant neurons and the total number of neurons for control and NL2A-overexpressing neurons. Aberrant neurons were defined as neurons with a cell body located outside of the GCL or displaying changes in their polarity. This ratio was not significantly altered by NL2A overexpression. $n_{animals}$=4.

Discussion

During their maturation, adult-born hippocampal neurons undergo massive death by apoptosis, suggesting a selection of the neurons that are the most relevant for the circuitry, and an elimination of neurons irrelevant for the network. However, the criteria for this selection are unclear. In the present study, we examined the cell-autonomous effects of the overexpression of different adhesion molecules on the development, synaptic integration and survival of adult-born neurons. We found that the adhesion molecules we studied had distinct effects on newborn neuron synaptogenesis. In particular, dnSynCAM1 decreased synaptic size while NL2A increased spine density, leading to decreased and increased newborn neuron survival, respectively. NL1B and SynCAM1 respectively increased spine density and spine size without increasing survival. Together, these results show that the manipulation of a single adhesion molecule is sufficient to modify synaptogenesis and/or synapse function, and to modify

newborn hippocampal neuron survival. This supports the hypothesis that synaptogenesis is important for neuronal survival, and that neurons that have an increased ability to connect to their pre- and post-synaptic partners have increased chances of survival.

Effects of synaptic adhesion molecules on the synaptogenesis and survival of adult-born hippocampal neurons

SynCAM1 overexpression did not increase glutamatergic synapse formation. This contradicts Robbins's *in vivo* findings, showing that *in vivo* SynCAM overexpression in CA1 neurons promoted excitatory synapse formation, and may be explained by differences in the experimental design. Indeed, as our retroviruses infect sparse neurons, we were able to overexpress the SynCAM1 in granule cells located far from each other. This approach allowed us to study the cell-autonomous effects of SynCAM1 overexpression, whereas Robbins and colleagues overexpressed SynCAM1 in all the excitatory neurons of the forebrain. Thus, it cannot be excluded that the increase in excitatory synapse density in CA1 neurons they observed *in vivo* is due to a non-cell-autonomous effect of SynCAM1 overexpression on CA1 neurons. Moreover, in line with our findings, SynCAM1 overexpression in cultured hippocampal neurons increases glutamatergic synapse efficacy without increasing glutamatergic synapse number [98].

As expected, NL1B overexpression increased spine density, which is in line with previous results [106,109]. Interestingly, SynCAM1 and NL1B overexpression had complementary effects: SynCAM1 overexpression selectively increased glutamatergic synapse and mossy fiber terminal maturation without having an effect on glutamatergic synapse density, whereas NL1B overexpression increased glutamatergic synapse density but did not affect synapse maturation. In both cases though, no effect on adult-born neuron survival could be detected.

However, expression of dnSynCAM1, the cytoplasmic tail of SynCAM1, which acts as a competitive inhibitor of SynCAM1 [95], decreased glutamatergic synapse and mossy fiber terminal maturation, and decreased newborn neuron survival. This suggests that synapse function is an important factor for newborn neuron survival, and that, if increasing synapse maturation by SynCAM1 overexpression is not sufficient to increase neuronal survival, decreasing synapse maturation by dnSynCAM1 expression does alter survival. Similarly, knockdown of NL1 in adult-born neurons has been shown to decrease adult-born neuron

survival [109]. Thus, if NL1 overexpression is not sufficient for increasing adult-born neuron survival, decreasing NL1 expression decreases adult-born granule cell survival. Thus, glutamatergic synapse formation is also an important factor for neuronal survival.

NL2A overexpression increased both glutamatergic and GABAergic synapse formation, confirming previous studies [106]. NL2A overexpression also had a small effect on glutamatergic synapse and mossy fiber terminal maturation, which has, to our knowledge, never been described. NL2A overexpression led to increased adult-born neuron survival, and this is remarkable because this implies that overexpression of a single adhesion molecule is sufficient to increase adult-born neuron survival by two-fold. NL2A only had transient effects on neuronal arborization and migration, suggesting that increasing glutamatergic and GABAergic synaptogenesis is by itself sufficient to increase adult-born neuron survival.

However, we cannot exclude a direct anti-apoptotic effect of NL2A on newborn neurons: NL2A could act directly on the apoptosis pathway to decrease the probability of neuronal death. However, to our knowledge, adhesion molecules have never been shown to play a role in the induction of apoptosis in physiological conditions, at least in the central nervous system. Additionally, NL2A and NL1B belong to the same family of proteins, and we did not observe any effect of NL1B overexpression on neuronal cell death. Thus, this seems unlikely.

In contrast, NL1B overexpression had no effect on adult-born neuron survival. According to the literature, NL1B overexpression does not increase GABAergic synaptogenesis. This leads to several hypotheses: 1. Increasing GABAergic synapse formation increases neuronal survival. 2. An increase in both GABAergic and glutamatergic synapse formation is necessary to increase neuronal survival. 3. NL2A leads to an increase in both synapse formation and maturation, which leads to increased neuronal survival. In any case, we can conclude that synapse formation and maturation play a role in neuronal survival.

Interestingly, changes in mossy fiber terminal maturation induced by the overexpression of the adhesion molecules we studied reflected changes in dendritic spine maturation: when dendritic spine maturation was modified, mossy fiber terminal maturation was modified to the same extent. This is in accordance with previous work showing that the development of dendritic inputs and axonal outputs is coordinated: they develop concomitantly on adult-born neurons [19,42,110]. Our results are also in line with the fact that SynCAM1 acts in developing hippocampal

neurons to shape migrating growth cones, and contributes to the differentiation of their axo-dendritic contacts [111]. Neuroligin-2 is a post-synaptic protein, so the fact that it influences, even slightly, mossy fiber terminal maturation, is puzzling. However, this could be explained by regulatory mechanisms synchronizing the development of the synaptic inputs and outputs of adult-born neurons. By increasing the maturation of dendritic spines, NL2 overexpression would then induce axon terminal maturation indirectly.

To confirm that synaptogenesis is involved in the survival of newborn neurons and complete our study, electrophysiological recordings of overexpressing newborn neurons in acute hippocampal slices will be essential. Indeed, our work did not allow to conclude whether the respective increase and decrease in synapse size we observed in SynCAM1-overexpressing neurons and dnSynCAM1-expressing neurons were related to a decrease in synaptic strength. Similarly, assessing whether the synapses produced by NL1B and NL2A newborn neurons are functional will be of interest to confirm that synaptogenesis influences neuronal survival. Indeed, one could imagine that NL1B and NL2A overexpression lead to the production of more dendritic spines, but that they do not form functional synapses. This would imply that NL2A has a direct effect on neuronal survival, and that it is not related to synaptogenesis. These electrophysiological analyses would also allow us to conclude whether overexpressing neurons are overall more or less synaptically active than control neurons, i.e. whether they have increased or decreased output to the circuitry. This knowledge is important, because, if the inputs the adult-born neurons receive may determine their survival, it is likely that the outputs they send to the network are also of importance for their survival.

Effects of synaptic adhesion molecules on the dendritic arborization and migration of adult-born hippocampal neurons

The overexpression of the adhesion molecules we studied had no long-lasting effects on the development of the dendritic arborization of adult-born neurons, and did not change newborn neuron dendritic extension at the timepoints we studied.

However, we found an increase in dendritic arborization complexity in adult-born neurons overexpressing NL2A at 14 dpi. These data are in line with the results of Ge and colleagues, who showed that eliminating excitatory GABA action on newborn hippocampal granule cells decreased dendritic arborization complexity at the same developmental timepoint [36]. Indeed, in

contrary to their approach, the overexpression of NL2A results in an increase in GABAergic synaptogenesis, which may increase excitatory GABA action. Together, these results suggest that GABA excitatory action may accelerate the development of the dendritic arborization.

In contrast, it is interesting to notice that, when we increased (SynCAM1 overexpression) or decreased glutamatergic action (dnSynCAM1 expression) by increasing or decreasing glutamatergic synapse maturation without affecting GABA excitatory action, we found a transient decrease and increase in dendritic arborization of adult-born neurons at 21 dpi, respectively, although the effect was small. Together, these results suggest that, while GABA excitatory action may accelerate the development of the dendritic arborization, glutamatergic action might delay it. This leads to the hypothesis that a good balance between GABA and glutamatergic synapses might be transiently necessary for the proper maturation of the dendritic arborization during adult-born neuron development, although much additional data will be necessary to validate this hypothesis.

Does the overexpression of adhesion molecules influence the fitness of adult-born neurons during synaptic competition?

Evidence for direct synaptic competition between cohorts of newborn neurons derives from the experiments of Tashiro and colleagues, in which the NMDA receptor subunit was specifically deleted in newborn neurons by retroviral mediated Cre-expression in mice with floxed NR1 alleles [78]. The authors used the same retroviral approach as us, allowing them to silence NMDA receptors in a sparse subset of adult-born neurons. When NMDA receptors were silenced only in a sparse subset of adult-born neurons, the survival of these adult-born neurons was decreased. However, when all the NMDAR were silenced pharmacologically in the DG, the survival of adult-born neurons was not compromised. This supports the notion that survival and integration of newborn neurons does not depend on the absolute, but rather on the relative degree of NMDA receptor activation.

Similarly, Kwon and colleagues showed that cortical neurons with higher levels of NL1 compared to their neighbours had increased spine density and functional synapse number, whereas neurons with lower levels of NL1 were deficient in the same aspects [103]. In contrary, global knockout of NL1 neither reduced functional glutamatergic synapse number nor dendritic

spine density of these neurons. Thus, during their maturation, hippocampal adult-born neurons may compete with other neurons for integration into the network and survival.

Synaptic competition may occur only between newborn neurons of the same age, but newborn neurons may also potentially compete with pre-existing, more mature, granule neurons. Yasuda and colleagues recently reported that the axons of genetically silenced granule cells become eliminated through competition with axons of unaffected cells of the same age [112]. Interestingly, this elimination was greatly reduced when post-natal neurogenesis was abolished, providing direct evidence for the possibility that mature neurons may also compete with young granule neurons for the activity-dependent refinement of DG-CA3 connections. However, given that the authors performed their experiments exclusively during postnatal stages, it remains to be established whether this activity-dependent competition between axons also operates in the adult DG.

Together, these studies support the hypothesis of a competition between adult-born neurons, or between adult-born neurons and more mature neurons, during the phase of survival. In this work, because of our experimental design, we genetically modified a sparse subset of adult neural progenitors, which gave rise to a sparse population of genetically modified adult-born neurons. Neurons expressing dnSynCAM1 may thus have decreased synaptic activity compared to their neighbours, whereas neurons overexpressing NL2A may have relatively increased synaptic activity. This might respectively give them a handicap or an advantage in their competition for survival with other granule cells, and might explain why dnSynCAM1 expression decreases, whereas NL2A overexpression increases, adult-born neuron survival.

Filopodia are thought to represent the first stage of synaptogenesis, and to develop into dendritic spines once they found a synaptic partner [113]. Overexpression of NL2A in newborn neurons slightly decreased the proportion of filopodia, suggesting that, in NL2A-overexpressing neurons, more filopodia found a partner, and consequently transformed into dendritic spines. This could be explained by the fact that, if a competition exists at the scale of the synapse, i.e. if several dendritic spines compete for the same axonal bouton in the case of a multiple synapse bouton, NL2A-overexpressing filopodia may have been favored in this competition: NL2A overexpression may have increased the competitivity of the synapses formed by these filopodia, for example by increasing their stability.

Does NL2A overexpression in adult-born neurons improve learning and memory?

NL2A overexpression increased newborn neuron survival between 14 and 28 dpi by two folds. Additionally, NL2A overexpression increased glutamatergic and GABAergic synapse density, and slightly increased glutamatergic synapse and axon terminal in adult-born neurons at 21 and 28 dpi. Thus, adult-born neurons overexpressing NL2A may display increased activity. Therefore, the hippocampus of mice injected with the virus carrying NL2A expression cassette contains a subpopulation of adult-born neurons with increased survival at the age when they have an impact on hippocampal LTP, and that potentially also display increased synaptic activity during the time window when newborn neurons have been shown to be crucial for memory formation. This leads to the hypothesis that mice injected with the virus carrying NL2A expression cassette may have increased learning and memory in complex behavioural tasks, as adult neurogenesis has been shown to be involved in learning performances.

Because of studies showing that epileptic activity leads to increased adult-born neuron maturation and survival, one could argue that accelerated maturation and increased survival of adult-born neurons are nefast for the function of the circuitry[114-116]. However, if NL2A-overexpressing neurons displayed increased maturation and increased survival, and migrated faster than controls, they did not migrate farther, as at 28 dpi, their migration was similar to controls. Also, at 14 dpi, the number of aberrant, i.e. neurons with a cell body located outside of the granule cell layer or displaying changes in their polarity, NL2A-overexpressing neurons and control neurons were comparable (Figure 49). Moreover, at 21 and 28 dpi, NL2A-overexpressing neurons diplayed similar neuronal arborization and dendritic extension as controls.

Thus, NL2A overexpression improves neuronal survival and augments synaptogenesis without leading to the production of more aberrant neurons, and does not have long-term effects on the migration, dendritic extension and arborization of adult-born neurons. It is thus unlikely that overexpressing NL2A in adult-born granule cells leads to the formation of adult-born neurons deleterious for the function of the circuitry, as it might be the case in epilepsy.

This means that overexpressing NL2A in adult-born granule cells may lead to the increased overall production of more active adult-born neurons, and therefore improve learning and

memory abilities, without deleterious consequences on the function of the network, as these neurons do not seem to be aberrantly connected. As a consequence, it would be of interest to test the learning and memory abilities of mice injected with a virus carrying NL2A-expression cassette. Adult-born granule cells have a specific role in learning and memory during the time window during which they display enhanced synaptic plasticity, i.e. around 28 dpi. Also, NL2A-overexpressing neurons show increased synaptogenesis at 21 and 28 dpi. Thus, the best timepoint to study learning and memory in these mice would be 28 days after retroviral injection.

The finding that NL2A increases newborn neuron survival is very interesting in terms of clinical approaches. Indeed, if mice overexpressing NL2A in adult-born neurons show increased learning and memory performances, it suggests that increasing the survival and the activity of newborn hippocampal neurons may relieve the symptoms of patients suffering from diseases such as Alzheimer's disease or depression. The next step would be to test the same approach in mouse models of these pathologies.

General discussion

Adult hippocampal neurogenesis leads to the continuous production of new granule cells in the hippocampus. These neurons born in adulthood result from a lengthy process of generation and

maturation, which has a heavy energetic cost. However, 70% of these neurons die at different steps of their maturation, before 4 weeks of maturation. The specific function of adult-born neurons seems limited to the time window during which they display increased plasticity, i.e. between 3 and 7 weeks of maturation, thus after the period of cell death. This suggests that adult-born neurons undergo a stringent selection before they accomplish their function in the network, and, as the energetic cost of adult-born neuron generation and maturation is high, that this selection may be crucial for proper functioning of the network. If this selection exists, on which criteria is it made?

In contrary to prenatal neurogenesis, adult neurogenesis occurs in a behaving animal, which constantly receives stimuli from its surrounding environment. Also, prenatal astrocytogenesis occurs after prenatal neurogenesis [117], whereas during adult neurogenesis, newborn neurons grow into a pre-existing network of astrocytes. Finally, adult-born neurons integrate into a pre-existing, fixed neuronal network, whereas it is not the case during prenatal neurogenesis. These peculiar conditions may have consequences on the modalities of synaptic integration and survival of adult-born neurons. In this work, we examined three different aspects of the synaptic integration of newborn hippocampal neurons: neuronal network activity, astrocytic ensheathment, and the cell-autonomous overexpression of synaptic adhesion molecules, and their effect on the maturation and survival of these neurons.

We found that propofol anesthesia significantly impaired the survival and dendritic maturation of adult-born neurons. Thus, a global increase in inhibition seems to be deleterious for the development and survival of adult-born neurons. This suggests that adult-born neuron maturation and survival depend on the activity of the surrounding, pre-existing, neuronal network. We discovered that pre-existing astrocytic processes ensheath the synapses formed by adult-born neurons very early in neuronal development, and that inhibition of astrocytic glutamate reuptake reduces postsynaptic currents and increases paired-pulse facilitation in adult-born neurons, suggesting that perisynaptic processes modulate synaptic transmission on these cells. This shows that, as adult-born neurons grow, their synapses are very quickly ensheathed by the astrocytic processes from surrounding, and that these astrocytic processes have a role in the functional and structural integration of adult-born neurons into the pre-existing neuronal network. Finally, we found that the manipulation of a single adhesion molecule is sufficient to modify synaptogenesis and/or synapse function, and to modify newborn hippocampal neuron survival. This suggests that synapse formation and maturation,

and more generally the ability of the newborn neuron to communicate with its synaptic partners, is crucial for its survival.

Effect of anesthesia on the maturation and survival of adult-born neurons

In this project, we found that adult-born mouse hippocampal neurons are vulnerable to propofol anesthesia, which reduced neuron survival and dendritic maturation *in vivo*. Propofol impaired the survival and maturation of adult-born neurons in an age-dependent manner. Anesthesia induced a significant decrease in the survival of neurons that were 17 days old at the time of anesthesia, but not of neurons that were 11 days old. Similarly, propofol anesthesia significantly reduced the dendritic maturation of neurons generated 17 days before anesthesia, without interfering with the maturation of neurons generated 11 days before anesthesia. These results point to a developmental stage-dependent impact of propofol anesthesia on differentiating adult-born hippocampal neurons.

Together, these data suggest that the activity of the neuronal network influences the survival and maturation of adult-born neurons. Indeed, propofol acts mainly as a GABAergic agonist: it binds to $GABA_A$ receptors and potentiates their activity by slowing the channel-closing time [118,119]. Thus, propofol anesthesia is expected to decrease the excitation/inhibition balance of the neuronal network, and thus to decrease global network activity, by increasing GABAergic transmission. However, propofol anesthesia may have other effects than increasing GABAergic transmission.

Indeed, propofol has also been shown to act as an NMDA receptor antagonist [120,121] and to decrease stimulated dopamine release via a mechanism independent of $GABA_A$ receptors [122]. Thus, although the main mechanism of action of propofol is an increase in GABAergic activity, propofol has other effects on synaptic transmission, independent of GABAergic activity. As a consequence, to confirm whether the decrease in maturation and survival we observed in adult-born hippocampal neurons is due to an actual increase in the global GABAergic activity of the network and not to other effects of propofol, it would be of interest to inject our mice with another GABAergic agonist, such as other anesthetics or sedatives, like benzodiazepines. Indeed, benzodiazepines such as diazepam act as positive allosteric modulators of $GABA_A$ receptors, by increasing the affinity of synaptic $GABA_A$ receptors for GABA and increasing the frequency of openings of extrasynaptic $GABA_A$ receptors [123].

Given that the survival and maturation of adult-born neurons are increased by stimulating external conditions such as environmental enrichment [24] and altered by impoverished environment such as social isolation [124-126], one can also argue that the effects of anesthesia on adult neurogenesis we observed are due to sleep itself, i.e. a decrease in the interactions with the environment, and not to a direct increase in global GABAergic transmission. To dissociate the effects induced by sleep and by global network inhibition, we could inject components increasing GABAergic transmission without inducing sleep, such as GABA transaminase inhibitors like valproic acid, which induce the accumulation of GABA at synapses, as GABA transaminase degrades GABA. Additionally, performing the reverse approach as in our study, by decreasing GABA transmission, or increasing glutamatergic transmission, to provoke an increase in the excitation/inhibition balance in the brain, and showing the opposite effect, i.e. an increase and the maturation and survival of newborn neurons, would add weight to our data. For this goal, we could use AMPAkines, which are chemical compounds stimulating AMPA receptors, or $GABA_A$ receptor antagonists such as flumazenil, which is used to reverse anesthesia in patients.

The differential effect of propofol anesthesia on 17 and 11-day-old neurons may be explained by two factors. First, the animals were all sacrified at 21 days after retroviral or BrdU injection, meaning that neurons that underwent anesthesia at 11 days of development were analyzed 8 days after, whereas neurons that underwent anesthesia at 17 days of development were analyzed only 4 days after. Thus, the fact that we could not detect an effect of anesthesia at 11 dpi in 21 days old newborn neurons could be explained by the fact that the effect is only transient and diseappears after 4 days: the maturation of adult-born neurons would then be simply delayed, and cell death only transiently increased. Consequently, anesthetizing mice at 11 dpi and sacrifying them at 15 dpi, and then anesthetizing mice at 17 dpi and sacrifying them at 25 dpi would allow to discriminate whether the absence of effect at 11 dpi is due to a difference in the developmental stage of newborn neurons, or simply due to the transient nature of the effect of anesthesia.

Secondly, if the differential effect of propofol anesthesia on 17 and 11-day-old neurons is actually due to a difference in the developmental stage of newborn neurons, it may be explained by the GABA shift occuring in adult-born neurons around 14 dpi: from being depolarizing, GABA becomes hyperpolarizing in newborn neurons. Thus, at 11 dpi, increasing global

GABAergic transmission may increase newborn neuron excitation and thus increase their activity, which does not affect maturation and survival. In contrast, at 17 dpi, this increase in global GABAergic transmission may increase the inhibition of newborn neurons and thus reduce their activity, leading to a decrease in their maturation and survival.

In the previous paragraphs, we assumed that the effect of propofol anesthesia is non-cell autonomous. However, our study does not allow us to determine whether the effect we observed on newborn neurons is due to a direct effect of anesthesia on newborn neurons, which may be more sensitive than pre-existing neurons to anesthesia as they are still immature, or to the actual decrease in the excitation/inhibition balance of the surrounding network, that acts indirectly on newborn neurons.

To answer this question, we could engineer a mouse expressing Designer Receptor Exclusively Activated by Designer Drugs (DREADDs) in forebrain neurons. DREADDs are engineered G-protein coupled receptors, which are activated by otherwise inert drug-like small molecules. These receptors allow the remote control of the activity of neurons in vivo. In particular, the activation of Hm3Dq by clozapine-N-oxide (CNO) in neurons activates Gq-mediated signaling and induces neuronal burst firing [127]. Conversely, the activation of Hm4Di in neurons by CNO induces Gi activation and neuronal silencing [128]. CNO can be administered by peripheral injection. Therefore, we could engineer mice expressing Hm4Di in forebrain neurons and graft wild-type adult neural stem cells in the dentate gyrus of these mice. Then, we would activate these receptors with peripheral injection of CNO at 11 dpi or 17 dpi, and assess newborn neuron maturation and survival at 21 dpi. If adult-born neuron survival and maturation are altered by this approach, this means that the sole inhibition of the surrounding neuronal network is sufficient to alter newborn neuron maturation and survival, and thus, that adult-born neuron maturation and survival depends on the global activity of the neuronal network. Conversely, performing exactly the same study with Hm3Dq would allow us to investigate whether a global increase in excitation increases the maturation and survival of newborn neurons. Expressing Hm4Di or Hm3Dq in adult-born neurons via our retroviral approach and assessing the outcome of the activation of these receptors at 11 dpi and 17 dpi may also shed light on an eventual direct, cell-autonomous effect of the inhibition or excitation of adult-born neurons at these developmental timepoints.

Adult neurogenesis is involved in learning and memory, and anesthesia is suspected to contribute to the onset of memory deficits in patients which underwent an operation. These deficits, that the elderly are more likely to develop, is known as postoperative cognitive dysfunction [129]. We showed a decrease in the survival of newborn neurons after anesthesia, potentially leading to a decrease in adult neurogenesis, if the proliferation of neuronal progenitors does not increase via a compensatory mechanism. This decrease in adult neurogenesis after anesthesia might link the memory deficits provoked by operations to anesthesia.

Indeed, a threshold in the number of newborn hippocampal neurons produced per day below which cognitive deficits appear may exist. If this is the case, as aging decreases the production of hippocampal newborn neurons, anesthesia, by further decreasing adult neurogenesis, may lead to cognitive deficits in the elderly. The fact that these deficits are more rarely observed in younger patients may be explained by the higher levels of neurogenesis in young humans: a decrease in adult neurogenesis has no visible effect on the cognitive performances of young humans, because the threshold in newborn neuron production below which deficits appear has not been reached. Therefore, the equivalent of environmental enrichment after an operation under anesthesia might help the elderly recover their learning and memory performances: one could think of preventing cognitive deficits in these patients by making them perform a variety of social, physical and intellectual activies after anesthesia, as this might help the levels of neurogenesis get back to pre-operation levels. Potentially, administering molecules increasing overall levels of adult neurogenesis after an operation may also be of great help for elderly patients to recover learning and memory performances comparable to those before anesthesia.

Astrocytic ensheathment of the synapses of adult-born neurons

In the second project, we found that the synapses of newborn neurons are ensheathed by astrocytic processes, and that this ensheathment appears very early: at 7 dpi, this ensheathment is already present on the dendrites of adult-born neurons. Additionally, this ensheathment does not change with the age of the neuron or the size of the synapse. Furthermore, inhibiting astrocytic glutamate re-uptake reduced postsynaptic currents and increased paired-pulse facilitation in adult-born neurons. This points to a modulation of synaptic transmission by perisynaptic astrocytic processes. Finally, we found that some astrocytic processes were

intercalated between dendritic spines from newborn neurons and potential presynaptic partners, suggesting that astrocytes may also play a structural role in the connectivity of the spines formed by adult-born neurons. Together, these results indicate that pre-existing astrocytes remodel their processes to ensheathe synapses of adult-born neurons and participate to the functional and structural integration of these cells into the hippocampal network.

For electrophysiological analyses, we applied dihydrokainate (DHK), which specifically blocks the astrocytic glutamate transporter GLT-1, on acute slices of C57BL6/J mice injected with a cag-GFP retrovirus 30 days before. We recorded post-synaptic responses of GFP-labeled newborn neurons evoked by paired pulses delivered to the perforant path. DHK reduced the EPSC amplitude and increased paired pulse ratio in newborn neurons. The enhanced paired pulse ratio indicates that presynaptic release probability was reduced by DHK. This showed that inhibiting astrocytic glutamate re-uptake decreases the presynaptic release probability at synapses formed by adult-born neurons. Thus, we concluded that astrocytic glutamate transporters enhance release probability and, as a consequence, strengthen excitatory synaptic transmission onto newly generated granule cells.

However, like any inhibitor, DHK could have non-specific effects: a study suggested that, in addition to inhibiting glutamate transport in astrocytes, DHK prevents astrocyte potassium uptake[130]. This is why performing the same experiment with other inhibitors of GLT-1, such as WAY-213,613, would further validate these results. Also, we could inhibit other functions of astrocytes, such as activity-dependent ATP release. Indeed, the ATP released by astrocytes as a result of neuronal activity can also participate in activity-dependent synaptic modulation: Zhang et al. showed that the release of ATP from astrocytes tonically suppresses glutamatergic synapses in hippocampal neurons in culture [131]. Thus, inhibition of ATP release might potentially affect the synaptic transmission of adult-born neurons, and this would strengthen our conclusion that astrocytic processes ensheathing adult-born neurons play an important role in their synaptic transmission.

The observation of astrocytic ensheathment of multiple synapse dendrites and boutons showed that the multiple synaptic partners involved in these structures shared a single astrocytic process in most cases, suggesting that astrocytes remodel their processes to accommodate the spines or mossy fiber terminals of adult-born neurons. The existence of multiple synapse dendrites (MSD) and boutons (MSB) suggests that adult-born neurons may compete with other neurons

for the access to their synaptic partners [19]. This peculiar astrocytic ensheathment on MSD and MSB suggests that astrocytes might play a role in this competition, by mechanically facilitating the access of the spines or the axon terminals of the newborn neuron to their synaptic partners, or, in contrary, by mechanically blocking the access to the partners. Astrocytic processes ensheathing these structures may also release factors attracting or repelling the dendritic spines or axonal boutons of the adult-born neuron. If astrocytes do not let the newborn neuron form synapses, the newborn neuron would then be unable to make connections with the network, and be eliminated.

Dendritic spines often have twisted necks and do not necessarily synapse with the nearest axonal bouton, suggesting that the choice of their presynaptic partner is not made randomly, but according to specific cues. We hypothetized that astrocytes have a role in choice of the synaptic partner of adult-born neurons. Indeed, filopodia, which are thought to be nascent spines, have been shown to be attracted by glutamate [132]. Moreover, astrocytes clear glutamate from the synaptic cleft and reduce its diffusion in the extrasynaptic space. Thus, nascent spines may tend to grow towards the least ensheathed bouton. In addition, astrocytic processes may physically block the contact of the spine with its potential presynaptic partners. Thus, astrocytes may physically and chemically guide nascent spines towards a specific presynaptic partner. Finally, by secreting molecules that induce synaptogenesis or synaptic plasticity, such as Thrombospondins [89], glypicans [133], D-serine [134], or ATP [135], astrocytes could induce synaptogenesis of adult-born neurons on selective synaptic partners.

We used serial section electron microscopy to study the boutons located close to dendritic spines from new neurons but not synapsing with them. We found that for 38% of these boutons, an astrocytic process was intercalated between the bouton and the new spine. These results show that pre-existing perisynaptic processes intercalate between the new spines and some of their potential presynaptic partners and, by doing so, might play a structural role in their connectivity by blocking the access of the spines to some of their potential partners. However, electron microscopy does not allow the analysis of the same spine over time, thus this approach becomes very quickly limited when it comes to answer this question.

To test these hypotheses, i.e. synaptic competition exists between newborn neurons and other newborn or pre-existing neurons, and astrocytes play a role in this competition by influencing the choice of the synaptic partner of adult-born neurons, time-lapse imaging would be a better

approach than electron microscopy. Indeed, with this technique, we could follow the formation of multiple synapse boutons and multiple synapse dendrites, and observe how astrocytic processes remodel when the spines or the mossy fiber terminals of adult-born neurons connect to their partners. However, organotypic slices are very difficult to make after P11. Also, labeling several presynaptic neurons and post-synaptic adult-born neurons and astrocytes with distinct colors would be very tricky, if not impossible. Thus, it would be hard to perform this experiment in the context of adult neurogenesis using organotypic slices.

This question of the choice of the synaptic partner and the role of astrocytes in it could be studied with another, more ambitious and recent *in vitro* technique: the three dimensional co-cultures of hippocampal neurons and astrocytes. Neurons within these structures exhibit complex 3-dimensional morphologies with rich neurite arborization, can be electrophysiologically recorded and display functional synapse formation and network properties [136]. In this model, grafting adult neural stem cells expressing RFP in an environment of pre-existing hippocampal neurons expressing GFP and pre-existing astrocytes expressing CFP (Cyan fluorescent protein) may allow to examine how astrocytic processes remodel during the formation of synapses from adult-born neurons. Indeed, we could perform two-photon time-lapse imaging on these structures and study the sequence of events of the formation of a synapse of newborn neurons, and how astrocytes remodel to let the new neurons in, or, in contrary, to block them from accessing their partners. However, the resolution limit of a two-photon microscope is around 0.5 µm, e.g. the size of a thin spine. As a consequence, this would be very difficult to perform for dendritic spines, but as mossy fiber terminals are larger, it might be feasible to study how they connect to the dendrites of other neurons and how whether and when astrocytic processes remodel during the formation of the synapse with this approach.

The next step of this project is to interfere with astrocytic function and to evaluate the outcome of this interference on adult-born neuronal synaptogenesis, maturation and survival. Sebastien Sultan, a post-doctoral trainee from Nicolas Toni's group, investigated the effect of blocking astrocytic exocytosis on the maturation and survival of adult-born hippocampal neurons. He found that blocking exocytosis from astrocytes reduced the formation of axo-spinous synapses with newborn neurons, but did not interfere with the stability of pre-existing synapses. Furthermore, blocking astrocytic exocytosis impaired the morphological and functional maturation of newborn neurons. These effects were mediated by D-serine, as intraperitoneal

injections of D-serine restored the maturation and the formation of dendritic protrusions by adult-born neurons. Finally, the survival of adult born hippocampal neurons was also altered by the blockade of astrocytic exocytosis.

These results combined to ours show that astrocytes have a prominent role in the morphological and functional maturation of newborn neurons, and that they also influence neuronal survival. Astrocytes may act at different levels to promote the survival of adult-born neurons. By reuptaking glutamate at the synaptic cleft, they enhance presynaptic release probability at the synapses formed by adult-born neurons, which may enhance adult-born neuron activity and thus increase their survival. By secreting molecules such as D-serine, astrocytes stimulate the synaptogenesis of adult-born neurons, which also may contribute to their survival. By blocking the spines of newborn neurons from accessing some of their potential presynaptic partners, astrocytes may allow newborn neurons to form relevant connections, and therefore to avoid elimination by apoptosis.

After acting on astrocytic release, we could modify other components of neuron-glia communication, and evaluate the outcome on adult-born neuronal maturation and survival. In the mouse hippocampus, signaling by Eph receptors and their cell surface-associated ephrin ligands has been implicated in synapse and spine formation [137-141]. In particular, the A-type EphA4 is expressed in astrocytic persynaptic processes, and interacts with Ephrin-A3, which is enriched on dendritic spines in hippocampal neurons [142] Activation of EphA4 by Ephrin-A3 induces spine retraction, whereas inhibition of EphA4/Ephrin-A3 interactions distorts spine shape and organization in hippocampal slices [142]. Furthermore, loss of ephrin-A3 or Ephrin-A4 affects LTP formation [143]. Thus, astrocytes use the Eph-ephrin system to shape spine morphology and synaptic function. Disrupting this system, by disrupting the interaction of EphA4/EphrinA3 in hippocampal newborn neurons, or, in contrast, enhancing it, would be another way to alter the neuron-glia communication in adult-born neurons, by modifying, this time, the adhesions between astrocytic processes and newborn neurons. We could use our retroviral approach to knock-down or overexpress Ephrin-A3 in adult-born neurons, by expressing a shRNA directed against Ephrin-A3 or overexpressing Ephrin-A3 in newborn neurons. To knock-down or overexpress EphA4 in astrocytes, we could use a lentivirus containing the expression cassette of EphA4 coupled to the expression of a fluorescent reporter, under the control of an astrocytic promoter such as GFAP or Aldh1l1.

Overexpressing adhesion molecules in adult-born neurons

In this project, we examined the cell-autonomous effects of the overexpression of different adhesion molecules on the development, synaptic integration and survival of adult-born neurons. We found that the adhesion molecules we studied had distinct effects on newborn neuron synaptogenesis. In particular, dnSynCAM1 decreased synaptic size while NL2A increased spine density, leading to decreased and increased newborn neuron survival, respectively. NL1B and SynCAM1 respectively increased spine density and spine size without increasing survival. Together, these results show that the manipulation of a single adhesion molecule is sufficient to modify synaptogenesis and/or synapse function, and to modify newborn hippocampal neuron survival. This supports the hypothesis that synaptogenesis has a crucial role for neuronal survival, and that neurons that have an increased ability to connect to their pre- and post-synaptic partners have increased chances of survival.

Our immunohistochemical analyses showed that, upon retroviral infection, SynCAM1 cytosolic tail is present in the whole cytoplasm of HEK 293T cells, whereas SynCAM1 is located at the plasma membrane (Figure 25). As a consequence, SynCAM1 cytosolic tail is expected to bind the cytosolic substrates of SynCAM1, but may also bind other cytosolic substrates unaccessible to SynCAM1, as SynCAM1 is exclusively located at the plasma membrane. This could lead to side effects not related to SynCAM1 inhibition. This is why our approach would nicely be completed by silencing SynCAM1 in adult-born neurons via an shRNA directed against SynCAM1. Incidentally, although neurons expressing dnSynCAM1 display a maturation comparable to controls in terms of migration, spine density, dendritic extension and arborization, we cannot exclude the fact that their decreased survival is due to the toxicity of dnSynCAM1 itself. To eliminate this hypothesis, we could co-overexpress dnSynCAM1 and SynCAM1 in newborn neurons, which, if the decrease in survival is due to the decrease in synapse maturation we observed and not to dnSynCAM1 toxicity, should restore survival comparable to controls.

SynCAM1 and NL1B overexpression had complementary effects: SynCAM1 overexpression increased glutamatergic synapse maturation without having an effect on glutamatergic synapse formation, whereas NL1B overexpression increased glutamatergic synapse density but did not affect synapse maturation. In both cases though, no effect on adult-born neuron survival could be detected. On the contrary, dnSynCAM1 expression in newborn neurons led to lower survival, and Schnell and colleagues showed in 2013 that NL1B knockdown in adult-born neurons also

decreased their survival [109]. Thus, glutamatergic synapse maturation and formation are important factors for newborn neuron survival, even if increasing them separately has no effect on newborn neuron survival. However, an increase in both glutamatergic synapse density and maturation may lead to increased survival. As a consequence, it would be of interest to co-overexpress SynCAM1 and NL1B in adult-born neurons and to measure their survival between 2 and 4 weeks of maturation. Co-overexpression of these two proteins will increase both the formation and maturation of glutamatergic synapses on newborn neurons, and this will help determine whether increasing glutamatergic synaptogenesis may improve survival. Co-overexpression of SynCAM1 and NL2A may also be useful to determine whether increasing synapse maturation may further improve the survival of NL2A-overexpressing adult-born neurons. Similarly, using AMPAkines, which stimulate AMPA receptors and thus increase the global excitation of the network, we could make an attempt to potentiate the effects of NL1B, NL2A or SynCAM1 overexpression on newborn neuron maturation and survival.

NL2 had the strongest effect on the synaptogenesis of adult-born granule cells, and was the sole adhesion molecule of our work capable to improve the survival of adult-born neurons. Therefore, it would be valuable to focus on this molecule in further studies. First, completing our study by expressing a shRNA direct against NL2A in newborn neurons would be valuable. More ambitious and exciting, creating a mouse line overexpressing NL2A in all hippocampal adult-born neurons would greatly help the study of the behavioural effects of NL2A overexpression in adult-born neurons, as the retroviral approach only allows the overexpression of NL2A in a sparse subset of adult-born neurons.

To this end, we could take advantage of mice expressing GFP under the GAD67 promoter, GAD67-GFP mice. Zhao et al. discovered in 2010 that, unexpectedly, GAD67-GFP mice selectively and transitorily express GFP in newborn dentate granule cells of the adult hippocampus [144]. The authors determined that GFP expression covered the whole developmental stage of newborn neurons, beginning within the first week of cell division and disappearing as newborn neurons mature, about 4 weeks post-mitotic. We could modify this mouse line to create GAD67-GFP-2A-NL2A mice, which overexpress NL2A in newborn hippocampal neurons from 1 to 4 weeks of maturation, i.e. during the time window of synaptogenesis.

This mouse line would allow analyses that are difficult to perform using a retroviral approach to overexpress NL2A in adult-born neurons. In young mice, NL2A overexpression might greatly increase neurogenesis, and potentially increase learning and memory performances. These mice could be tested with different behavioural tasks assessing aspects of learning and memory related to adult hippocampal neurogenesis, such as spatial learning and pattern separation. As adult neurogenesis has been shown to be implicated in the pathogenesis of anxiety and depression [145-147], these mice could also be tested for these two parameters with elevated plus maze, the open field tests, that are testing anxiety-related behaviour, and forced swim test, testing depression-related behaviour. As a complement, it would be exciting to test whether animals overexpressing NL2A in adult-born neurons are more resistant to social isolation and stress such as chronic restraint stress, as these two parameters decrease adult neurogenesis and are known to induce anxiety and depression-related behaviours in rodents [148,149].

Sahay et al. ablated Bax in adult neural stem cells to induce the expansion of the population of adult-born neurons by suppressing newborn neuron death during their maturation, and showed an improvement in pattern separation, but no effect on other types of learning such as object recognition, spatial learning, contextual fear conditioning and extinction learning, and no behavioural response like that induced by anxiolytic agents or antidepressants [150].

However, if the hypothesis that adult-born neurons undergo a selection during which neurons unable to properly integrate into the circuitry are suppressed before 4 weeks of maturation is true, then the authors suppressed the hypothetical competition between adult-born neurons and other neurons, and allowed neurons that are weakly connected or not connected to the pre-existing network to survive. In contrary, if this selection exists, our approach should keep this selective survival intact, because it should improve newborn neuron survival by increasing the ability of new neurons to connect to the network, instead of suppressing their ability to be eliminated from it. In other words, at 4 weeks of maturation, NL2A-overexpressing neurons survive better because they passed the phase of competition and selection successfully, and not because this phase was suppressed. Thus, our study may shed light on the role of the phase of intense cell death that adult-born neurons undergo during their maturation. NL2A-overexpressing neurons may also have increased synaptic plasticity, which should be verified by electrophysiological analyses, and, as synaptic plasticity is one of the important neurochemical foundations of learning, this increased plasticity and improved survival of

newborn neurons may have great effects on learning and memory performances of GAD67-GFP-2A-NL2A mice.

Synaptic plasticity, adult neurogenesis and learning and memory performances have been shown to decrease during aging by numerous studies [151,152]. Similarly, Alzheimer's disease is characterized by memory loss, and data from mouse models show that adult neurogenesis and synaptic plasticity are altered in this disease. NL2A overexpression in adult-born hippocampal newborn neurons in aged mice may restore adult neurogenesis levels, and additionally lead to the production of more plastic adult-born neurons. Therefore, one may expect an improvement in learning and memory performances in aged mouse overexpressing NL2A in adult-born hippocampal neurons. Similarly, restoring adult neurogenesis and allowing the production of more plastic hippocampal newborn neurons by overexpression of NL2A in newborn neurons of Alzheimer's disease mouse models such as APP/PS1 mice and assessing the outcome on learning and memory capacities is of great interest in terms of clinical applications.

In this study, although in some cases we observed morphological changes of the axons and mossy fiber terminals of adult-born neurons overexpressing an adhesion molecule, we aimed at modifying the post-synaptic properties of newborn neurons to increase or decrease the synaptic input they receive. However, the output new neurons send to the network may be equally important for their selection.

Therefore, we could express Hm3Dq or Hm4Di using the same retroviral vector we used for overexpressing adhesion molecules in newborn hippocampal neurons, activate these receptors with peripheral injection of CNO during different time windows of development, and assess newborn neuron maturation and survival. The most interesting time window to increase or decrease firing in newborn neurons is the period of survival of adult-born neurons, i.e. before 4 weeks of maturation. We expect that during this time window Hm3Dq activation will lead to an increase in neuronal firing rate, and as increased adult-born neuronal activity may increase adult-born neuron survival, to increased maturation and survival of adult-born neurons. Hm4Di activation will presumably decrease neuronal firing rate and might lead to a decrease in newborn neuron maturation and survival.

Conclusions and perspectives

The work of my PhD suggests that intrinsic and extrinsic factors are involved in adult-born neuron survival: the activity of the network, the formation of functional astrocytic processes around the synapses of adult-born neurons, and the ability of newborn neurons to form functional synapses seem crucial for the further survival of adult-born neurons (Figure 50).

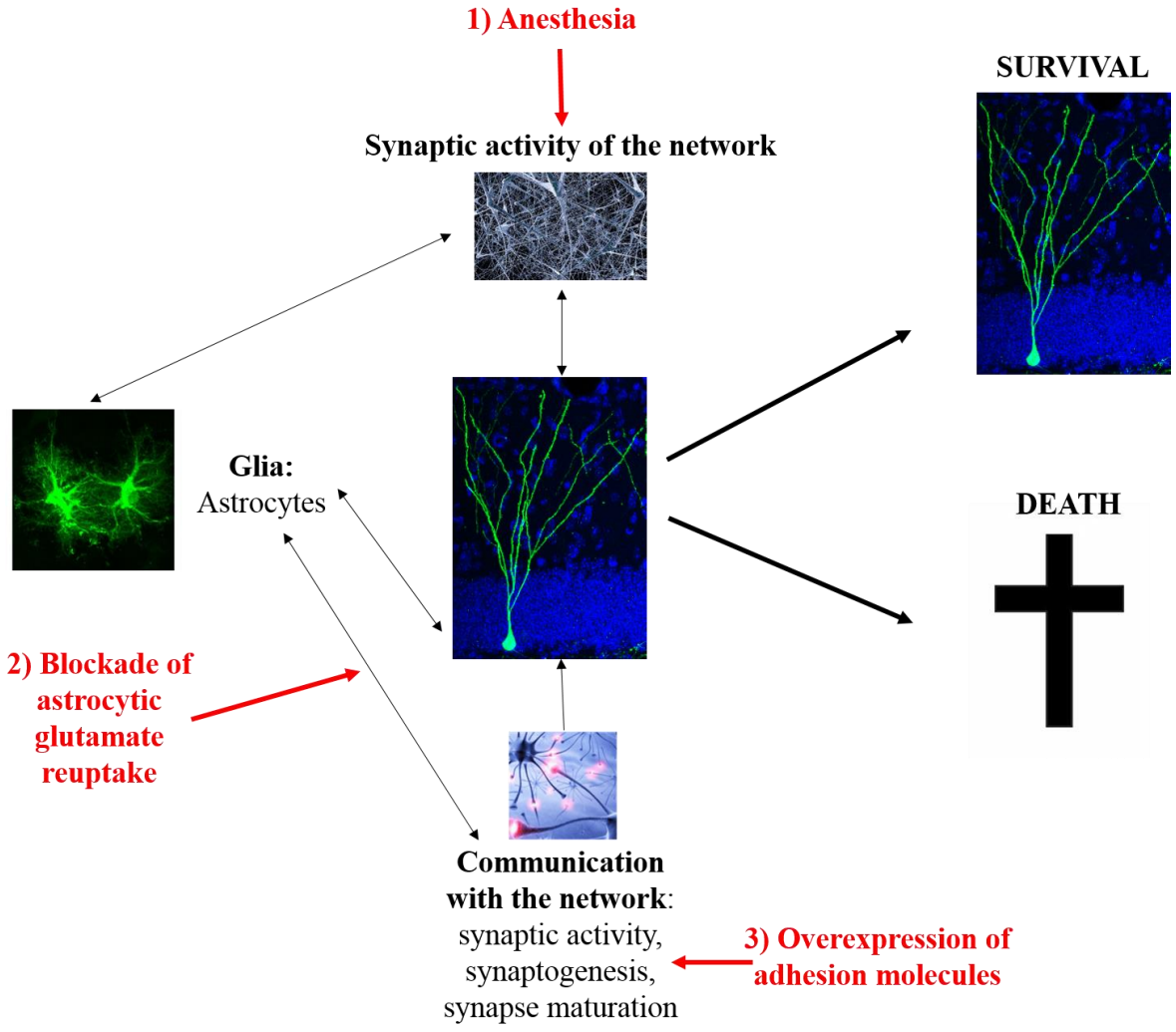


Figure 50: The synaptic integration and survival of adul-born neurons are influenced by intricated extrinsic (activity of the network, astrocytes) and intrinsic factors (synaptogenesis and synaptic activity of the neurons). In red, the manipulations we performed: 1) Anesthesia: Propofol anesthesia decreased newborn neuron survival and maturation. 2) Perisynaptic processes were present at the synapses of newborn neurons and blocking glutamate reuptake reduced synaptic transmission on new neurons. 3) Overexpression of adhesion molecules: The overexpression of a single adhesion molecule in newborn neurons was sufficient to increase synaptogenesis and survival of these neurons. The arrows show that these factors are interdependent. For example, astrocytes influence the

communication between newborn neurons and the rest of the network, and the synaptic activity of the network can modify the activity of astrocytes.

These factors all play a role in the communication of adult-born neurons with their surrounding environment, i.e. neighbouring astrocytes and neurons. Thus, our data suggest that increasing the ability of newborn neurons to communicate with the rest of the network, i.e. to receive information from neighbouring neurons or to send information to them, increases their survival. Similarly, we can hypothesize that newborn neurons unable to communicate with the rest of the network, i.e. unable to receive information from neighbouring neurons or to send information to them, are eliminated by apoptosis.

This elimination may be crucial for the proper function of the network, and a dysfunction of this selection might be illustrated by pathologies such as epilepsy. Indeed, Jessberger and colleagues showed that epilepsy increases the maturation and survival of adult-born hippocampal neurons, and leads to a high number of aberrantly connected newborn neurons [114]. These aberrant neurons may interfere with hippocampal function and contribute to cognitive impairment caused by epileptic activity in the hippocampus [115]. Several reviews also suggest that these aberrant neurons may increase the risk of epileptic seizures, which induces a vicious circle, in which epilepsy leads to the production of aberrant neurons, which further increase the risk of epileptic seizures, leading to chronic epilepsy [116]. In this disease, the selection of adult-born neurons may be abolished by the increased activity of the network. As aberrant neurons are not eliminated anymore, they have detrimental effects on the rest of the network. This also illustrates the bidirectional relationship between the newborn neurons and the pre-existing network: newborn neurons have an effect on the function of the network, and, in turn, the network may influence newborn neuron survival by a feedback regulatory mechanism.

In addition, our work showed that the regulation of the synaptic integration and survival of adult-born neurons takes place at many levels: at the level of the neuron itself, but also at the level of the cells of the network, i.e. neighbouring neurons and astrocytes. It is important to note that these levels are intricated (Figure 51): for instance, astrocytes have a role in newborn neuron synaptic transmission, but they also regulate the synaptic transmission of the other neurons of the network with the release of gliotransmitters, and thus influence the synaptic activity of the network [153].

In addition, parameters not related to synaptic communication, such as metabolic parameters, may also influence the selective survival of adult-born neurons. Indeed, Chichung Lie's group showed that impaired mitochondrial function altered the survival of newborn neurons whereas it only had transient effects on the parameters of newborn neuron synaptogenesis they measured [154]. This highlights that, *per se*, functional metabolism of adult-born neurons may be crucial for their survival.

In line with this, proper neurovascular coupling may be of importance for the survival of adult-born neurons. Indeed, neurovascular coupling links neuronal activity to cerebral blood flow, which is primordial for coupling the supply of oxygen and nutrients to neurons and neuronal activity, as the need for oxygen and nutrients increases with neuronal activity. Astrocytes play a key role in neurovascular coupling [155](Petzold 2011). Indeed, the release of glutamate by presynaptic neurons in the synaptic cleft elicits the production and release of prostaglandin E_2 (PGE_2) in the astrocytic process ensheathing the synapse. PEG_2 release leads to the dilation of blood vessels, leading to a local augmentation of cerebral blood flow. Thus, neurovascular coupling is another potential important factor for survival, which depends, at least in part, on astrocytic function.

In addition, microglia may also be involved in newborn neuron selection. Indeed, microglia has been shown to have a role in activity-dependent synaptic pruning [156-158]. Microglia also phagocyte the newborn neurons that died by apoptosis [159], and activated microglia inhibits the proliferation of neuronal precursor cells [160]. Thus, microglia and adult hippocampal neurogenesis are linked, but the effect of microglia on the survival of newborn neurons remains to be determined. The fact that microglia is involved in synaptic pruning suggests that microglia has a role in the elimination of non-functional synapses. Microglia might act after astrocytes in the selection of newborn neurons: astrocytes may select the synapses that are formed and persist, and microglia may eliminate non-functional synapses. Microglia might also eliminate synapses formed with the wrong partners.

Neurotrophic factors also promote adult-born neuron survival: among them, BDNF: normal BDNF signaling is required for the survival of adult-born neurons [161,162]. BDNF is released by neurons, glia, but also endothelial cells, and has different actions on these cells. Thus, the release of neurotrophic factors in the extracellular environment by different cell types adds a level of complexity to the regulation of newborn neuron survival and integration.

Last but not least, the role of timing has been shown to be crucial in adult-born neuron survival: when the neurons are formed determines whether they die or survive. We showed that newborn neurons displayed decreased survival when the mice were anesthetized at 17 dpi, whereas no effect was observed at 11 dpi. Additionally, Anderson et al. showed that associative learning increases the survival of hippocampal adult-born neurons only during a critical period: neurons aged of 1 to 2 weeks at the time of training displayed increased survival after learning, whereas cells that were younger or older did not [163]. Thus, the time when the neurons are generated determines whether they survive, and this highlights the fact that the survival of adult-born neurons depends on the needs of the network. Also, only few of the neurons that are produced daily survive, implying that the demands of the network may change within very short periods of time, and that a decrease or an increase in the survival of adult-born neurons coupled to variations in their production may allow a very quick adaptation to the needs of the network.

Therefore, many extrinsic and intrinsic factors influence neuronal survival and might play a role in their selection. These factors are interdependent, which may permit a fine regulation of the survival of adult-born neurons and of their incorporation in the network (Figure 51). However, this selection is still hypothetical, and the criteria of survival of adult-born neurons remain unclear.

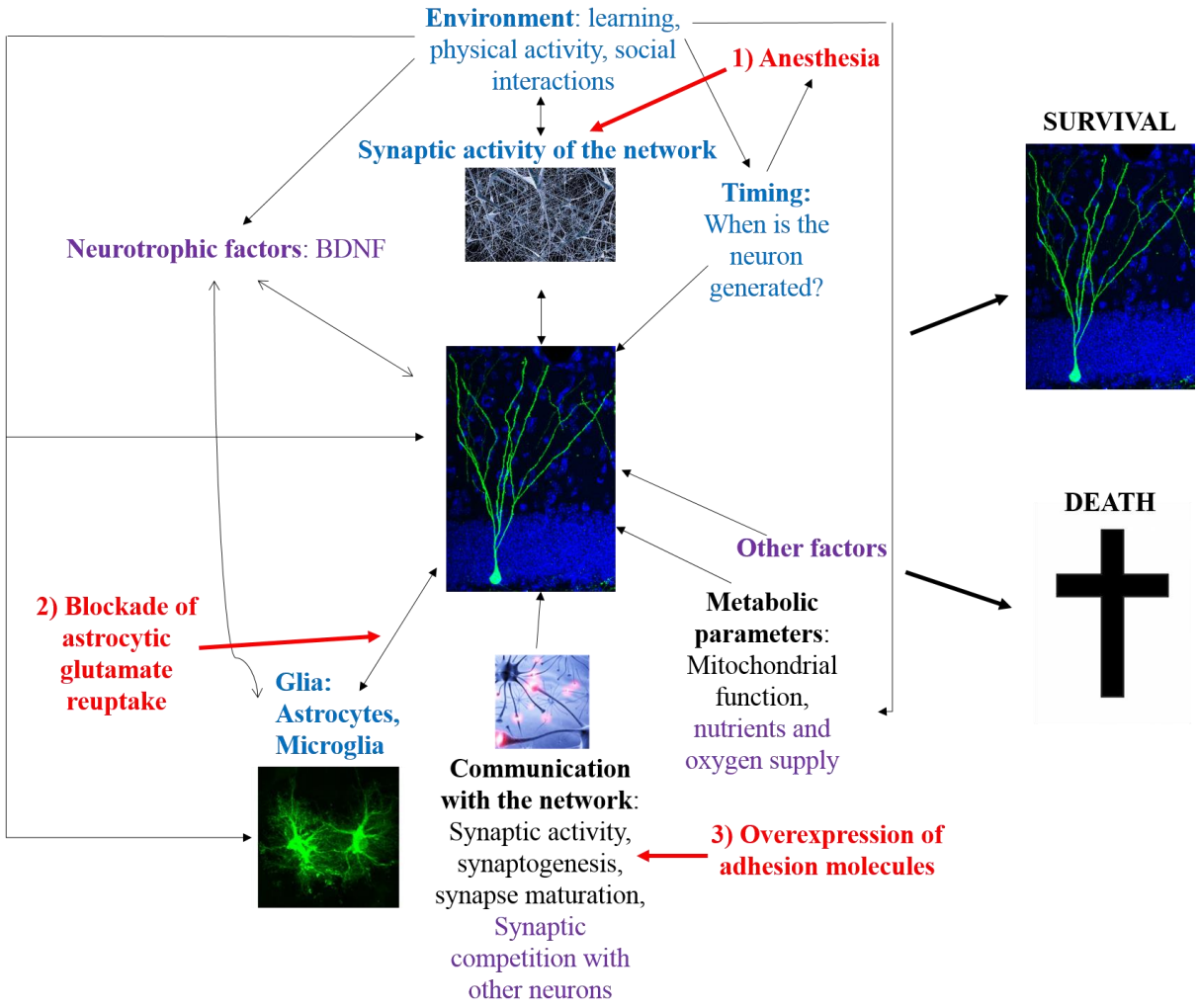


Figure 51: Potential factors influencing the choice between survival and death of newborn neurons. In blue: extrinsic factors; in black: intrinsic factors; in purple: factors that may be both intrinsic and extrinsic. In red, the manipulations we performed: 1) Anesthesia: Propofol anesthesia decreased newborn neuron survival and maturation. 2) Blockade of astrocytic glutamate reuptake: Perisynaptic processes were present at the synapses of newborn neurons and had a role in synaptic transmission. 3) Overexpression of adhesion molecules: The genetic modification of a single adhesion molecule in newborn neurons was sufficient to produce modifications in synaptogenesis and survival of these neurons. The arrows show that these factors are interdependent. For example, astrocytes influence the communication between newborn neurons and the rest of the network, and the synaptic activity of the network can modify the activity of astrocytes.

Understanding whether adult-born neurons are selected, and if yes, by which modalities, is crucial in terms of therapeutical applications. First, this is essential for finding the more relevant way to provoke a long-term increase in adult-born neuron survival and overall adult

neurogenesis in patients. Indeed, increasing adult hippocampal neurogenesis may be an efficient way to restore a number of granule cells in patients suffering from Alzheimer's disease or Huntington's disease, in the hope of improving their symptoms. It could also be of interest in neuropsychiatric diseases such as depression and schizophrenia, as adult neurogenesis has been shown to be altered in these pathologies.

More generally, understanding how the neurogenic niche works and which factors are important for the generation, maturation and survival of neurons is crucial to be able to maybe, one day, replace neurons in any region of the brain. Indeed, adult neural stem cells have been isolated from diverse brain areas, including non-neurogenic areas, suggesting that they are present in the whole CNS [164]. Understanding by which factors they start to produce new neurons, and what determines the subsequent survival of these neurons in the hippocampus, may allow to recreate a favorable environment for neurogenesis in other parts of the brain, which has great therapeutic implications.

References

1 http://stemcells.nih.gov/info/basics/pages/basics4.aspx.

2 Altman, J. Autoradiographic investigation of cell proliferation in the brains of rats and cats. *The Anatomical record* **145**, 573-591 (1963).

3 Altman, J. & Das, G. D. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. *The Journal of comparative neurology* **124**, 319-335 (1965).

4 Goldman, S. A. & Nottebohm, F. Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. *Proceedings of the National Academy of Sciences of the United States of America* **80**, 2390-2394 (1983).

5 Zupanc, G. K. Neurogenesis and neuronal regeneration in the adult fish brain. *Journal of comparative physiology. A, Neuroethology, sensory, neural, and behavioral physiology* **192**, 649-670, doi:10.1007/s00359-006-0104-y (2006).

6 Garcia-Verdugo, J. M. *et al.* The proliferative ventricular zone in adult vertebrates: a comparative study using reptiles, birds, and mammals. *Brain research bulletin* **57**, 765-775 (2002).

7 Amrein, I. & Lipp, H. P. Adult hippocampal neurogenesis of mammals: evolution and life history. *Biology letters* **5**, 141-144, doi:10.1098/rsbl.2008.0511 (2009).

8 Spalding, K. L. *et al.* Dynamics of hippocampal neurogenesis in adult humans. *Cell* **153**, 1219-1227, doi:10.1016/j.cell.2013.05.002 (2013).

9 Eckenhoff, M. F. & Rakic, P. Nature and fate of proliferative cells in the hippocampal dentate gyrus during the life span of the rhesus monkey. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **8**, 2729-2747 (1988).

10 Dayer, A. G., Ford, A. A., Cleaver, K. M., Yassaee, M. & Cameron, H. A. Short-term and long-term survival of new neurons in the rat dentate gyrus. *The Journal of comparative neurology* **460**, 563-572, doi:10.1002/cne.10675 (2003).

11 Bergmann, O. *et al.* The age of olfactory bulb neurons in humans. *Neuron* **74**, 634-639, doi:10.1016/j.neuron.2012.03.030 (2012).

12 Alvarez-Buylla, A., Garcia-Verdugo, J. M. & Tramontin, A. D. A unified hypothesis on the lineage of neural stem cells. *Nature reviews. Neuroscience* **2**, 287-293, doi:10.1038/35067582 (2001).

13 Lee, D. A. *et al.* Tanycytes of the hypothalamic median eminence form a diet-responsive neurogenic niche. *Nature neuroscience* **15**, 700-702, doi:10.1038/nn.3079 (2012).

14 Sanai, N. *et al.* Corridors of migrating neurons in the human brain and their decline during infancy. *Nature* **478**, 382-386, doi:10.1038/nature10487 (2011).

15 Ernst, A. *et al.* Neurogenesis in the striatum of the adult human brain. *Cell* **156**, 1072-1083, doi:10.1016/j.cell.2014.01.044 (2014).

16 Eriksson, P. S. *et al.* Neurogenesis in the adult human hippocampus. *Nature medicine* **4**, 1313-1317, doi:10.1038/3305 (1998).

17 Knoth, R. *et al.* Murine features of neurogenesis in the human hippocampus across the lifespan from 0 to 100 years. *PloS one* **5**, e8809, doi:10.1371/journal.pone.0008809 (2010).

18 Laplagne, D. A. *et al.* Functional convergence of neurons generated in the developing and adult hippocampus. *PLoS biology* **4**, e409, doi:10.1371/journal.pbio.0040409 (2006).

19 Toni, N. *et al.* Synapse formation on neurons born in the adult hippocampus. *Nature neuroscience* **10**, 727-734, doi:10.1038/nn1908 (2007).

20 Fred H. Gage, G. K., Hongjun Song. *Adult neurogenesis*. (Cold Spring Harbor Laboratory Press, 2008).

21 Toni, N. & Sultan, S. Synapse formation on adult-born hippocampal neurons. *The European journal of neuroscience* **33**, 1062-1068, doi:10.1111/j.1460-9568.2011.07604.x (2011).

22 Zhao, C., Deng, W. & Gage, F. H. Mechanisms and functional implications of adult neurogenesis. *Cell* **132**, 645-660, doi:10.1016/j.cell.2008.01.033 (2008).

23 Kohler, S. J., Williams, N. I., Stanton, G. B., Cameron, J. L. & Greenough, W. T. Maturation time of new granule cells in the dentate gyrus of adult macaque monkeys exceeds six months. *Proceedings of the National Academy of Sciences of the United States of America* **108**, 10326-10331, doi:10.1073/pnas.1017099108 (2011).

24 Kempermann, G., Gast, D., Kronenberg, G., Yamaguchi, M. & Gage, F. H. Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. *Development* **130**, 391-399 (2003).

25 Kempermann, G., Brandon, E. P. & Gage, F. H. Environmental stimulation of 129/SvJ mice causes increased cell proliferation and neurogenesis in the adult dentate gyrus. *Current biology : CB* **8**, 939-942 (1998).

26 Malberg, J. E., Eisch, A. J., Nestler, E. J. & Duman, R. S. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **20**, 9104-9110 (2000).

27 van Praag, H. *et al.* Functional neurogenesis in the adult hippocampus. *Nature* **415**, 1030-1034, doi:10.1038/4151030a (2002).

28 Zhao, C., Teng, E. M., Summers, R. G., Jr., Ming, G. L. & Gage, F. H. Distinct morphological stages of dentate granule neuron maturation in the adult mouse hippocampus. *The Journal*

of neuroscience : the official journal of the Society for Neuroscience **26**, 3-11, doi:10.1523/JNEUROSCI.3648-05.2006 (2006).

29 Song, J. *et al.* Parvalbumin interneurons mediate neuronal circuitry-neurogenesis coupling in the adult hippocampus. *Nature neuroscience* **16**, 1728-1730, doi:10.1038/nn.3572 (2013).

30 Ben-Ari, Y. Excitatory actions of gaba during development: the nature of the nurture. *Nature reviews. Neuroscience* **3**, 728-739, doi:10.1038/nrn920 (2002).

31 Overstreet Wadiche, L., Bromberg, D. A., Bensen, A. L. & Westbrook, G. L. GABAergic signaling to newborn neurons in dentate gyrus. *Journal of neurophysiology* **94**, 4528-4532, doi:10.1152/jn.00633.2005 (2005).

32 Tozuka, Y., Fukuda, S., Namba, T., Seki, T. & Hisatsune, T. GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. *Neuron* **47**, 803-815, doi:10.1016/j.neuron.2005.08.023 (2005).

33 Markwardt, S. J., Dieni, C. V., Wadiche, J. I. & Overstreet-Wadiche, L. Ivy/neurogliaform interneurons coordinate activity in the neurogenic niche. *Nature neuroscience* **14**, 1407-1409, doi:10.1038/nn.2935 (2011).

34 Ge, S., Pradhan, D. A., Ming, G. L. & Song, H. GABA sets the tempo for activity-dependent adult neurogenesis. *Trends in neurosciences* **30**, 1-8, doi:10.1016/j.tins.2006.11.001 (2007).

35 Karten, Y. J., Jones, M. A., Jeurling, S. I. & Cameron, H. A. GABAergic signaling in young granule cells in the adult rat and mouse dentate gyrus. *Hippocampus* **16**, 312-320, doi:10.1002/hipo.20165 (2006).

36 Ge, S. *et al.* GABA regulates synaptic integration of newly generated neurons in the adult brain. *Nature* **439**, 589-593, doi:10.1038/nature04404 (2006).

37 Chancey, J. H. *et al.* GABA depolarization is required for experience-dependent synapse unsilencing in adult-born neurons. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **33**, 6614-6622, doi:10.1523/JNEUROSCI.0781-13.2013 (2013).

38 Piatti, V. C. *et al.* The timing for neuronal maturation in the adult hippocampus is modulated by local network activity. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **31**, 7715-7728, doi:10.1523/JNEUROSCI.1380-11.2011 (2011).

39 Mongiat, L. A., Esposito, M. S., Lombardi, G. & Schinder, A. F. Reliable activation of immature neurons in the adult hippocampus. *PloS one* **4**, e5320, doi:10.1371/journal.pone.0005320 (2009).

40 Esposito, M. S. *et al.* Neuronal differentiation in the adult hippocampus recapitulates embryonic development. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **25**, 10074-10086, doi:10.1523/JNEUROSCI.3114-05.2005 (2005).

41 Nicolas Toni, A. S. in *Adult neurogenesis* (in press).

42 Toni, N. *et al.* Neurons born in the adult dentate gyrus form functional synapses with target cells. *Nature neuroscience* **11**, 901-907, doi:10.1038/nn.2156 (2008).

43 Schmidt-Hieber, C., Jonas, P. & Bischofberger, J. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature* **429**, 184-187, doi:10.1038/nature02553 (2004).

44 Ge, S., Yang, C. H., Hsu, K. S., Ming, G. L. & Song, H. A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. *Neuron* **54**, 559-566, doi:10.1016/j.neuron.2007.05.002 (2007).

45 Gu, Y. *et al.* Optical controlling reveals time-dependent roles for adult-born dentate granule cells. *Nature neuroscience* **15**, 1700-1706, doi:10.1038/nn.3260 (2012).

46 Kempermann, G., Kuhn, H. G. & Gage, F. H. More hippocampal neurons in adult mice living in an enriched environment. *Nature* **386**, 493-495, doi:10.1038/386493a0 (1997).

47 van Praag, H., Kempermann, G. & Gage, F. H. Running increases cell proliferation and neurogenesis in the adult mouse dentate gyrus. *Nature neuroscience* **2**, 266-270, doi:10.1038/6368 (1999).

48 van Praag, H., Christie, B. R., Sejnowski, T. J. & Gage, F. H. Running enhances neurogenesis, learning, and long-term potentiation in mice. *Proceedings of the National Academy of Sciences of the United States of America* **96**, 13427-13431 (1999).

49 Pacteau, C., Einon, D. & Sinden, J. Early rearing environment and dorsal hippocampal ibotenic acid lesions: long-term influences on spatial learning and alternation in the rat. *Behavioural brain research* **34**, 79-96 (1989).

50 Wainwright, P. E., Levesque, S., Krempulec, L., Bulman-Fleming, B. & McCutcheon, D. Effects of environmental enrichment on cortical depth and Morris-maze performance in B6D2F2 mice exposed prenatally to ethanol. *Neurotoxicology and teratology* **15**, 11-20 (1993).

51 Kempermann, G., Kuhn, H. G. & Gage, F. H. Experience-induced neurogenesis in the senescent dentate gyrus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **18**, 3206-3212 (1998).

52 Clelland, C. D. *et al.* A functional role for adult hippocampal neurogenesis in spatial pattern separation. *Science* **325**, 210-213, doi:10.1126/science.1173215 (2009).

53 Alonso, M. *et al.* Activation of adult-born neurons facilitates learning and memory. *Nature neuroscience* **15**, 897-904, doi:10.1038/nn.3108 (2012).

54 Chow, B. Y. *et al.* High-performance genetically targetable optical neural silencing by light-driven proton pumps. *Nature* **463**, 98-102, doi:10.1038/nature08652 (2010).

55 Curtis, M. A. *et al.* Increased cell proliferation and neurogenesis in the adult human Huntington's disease brain. *Proceedings of the National Academy of Sciences of the United States of America* **100**, 9023-9027, doi:10.1073/pnas.1532244100 (2003).

56 Curtis, M. A., Waldvogel, H. J., Synek, B. & Faull, R. L. A histochemical and immunohistochemical analysis of the subependymal layer in the normal and Huntington's disease brain. *Journal of chemical neuroanatomy* **30**, 55-66 (2005).

57 Batista, C. M. *et al.* A progressive and cell non-autonomous increase in striatal neural stem cells in the Huntington's disease R6/2 mouse. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **26**, 10452-10460, doi:10.1523/JNEUROSCI.2850-06.2006 (2006).

58 Lazic, S. E. *et al.* Decreased hippocampal cell proliferation in R6/1 Huntington's mice. *Neuroreport* **15**, 811-813 (2004).

59 Gil, J. M. *et al.* Reduced hippocampal neurogenesis in R6/2 transgenic Huntington's disease mice. *Neurobiology of disease* **20**, 744-751, doi:10.1016/j.nbd.2005.05.006 (2005).

60 van Dellen, A., Blakemore, C., Deacon, R., York, D. & Hannan, A. J. Delaying the onset of Huntington's in mice. *Nature* **404**, 721-722, doi:10.1038/35008142 (2000).

61 Rodriguez, J. J. & Verkhratsky, A. Neurogenesis in Alzheimer's disease. *Journal of anatomy* **219**, 78-89, doi:10.1111/j.1469-7580.2011.01343.x (2011).

62 Ziabreva, I. *et al.* Altered neurogenesis in Alzheimer's disease. *Journal of psychosomatic research* **61**, 311-316, doi:10.1016/j.jpsychores.2006.07.017 (2006).

63 Jin, K. *et al.* Increased hippocampal neurogenesis in Alzheimer's disease. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 343-347, doi:10.1073/pnas.2634794100 (2004).

64 Jucker, M. The benefits and limitations of animal models for translational research in neurodegenerative diseases. *Nature medicine* **16**, 1210-1214, doi:10.1038/nm.2224 (2010).

65 Taupin, P. Neurogenesis and Alzheimer's disease. *Drug target insights* **1**, 1-4 (2006).

66 Kaneko, N. & Sawamoto, K. Adult neurogenesis and its alteration under pathological conditions. *Neuroscience research* **63**, 155-164, doi:10.1016/j.neures.2008.12.001 (2009).

67 Czeh, B. *et al.* Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. *Proceedings of the National Academy of Sciences of the United States of America* **98**, 12796-12801, doi:10.1073/pnas.211427898 (2001).

68 Malberg, J. E. & Duman, R. S. Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. *Neuropsychopharmacology : official*

publication of the American College of Neuropsychopharmacology **28**, 1562-1571, doi:10.1038/sj.npp.1300234 (2003).

69 Duman, R. S., Nakagawa, S. & Malberg, J. Regulation of adult neurogenesis by antidepressant treatment. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology* **25**, 836-844, doi:10.1016/S0893-133X(01)00358-X (2001).

70 Santarelli, L. *et al.* Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**, 805-809, doi:10.1126/science.1083328 (2003).

71 Reif, A. *et al.* Neural stem cell proliferation is decreased in schizophrenia, but not in depression. *Molecular psychiatry* **11**, 514-522, doi:10.1038/sj.mp.4001791 (2006).

72 Duan, X. *et al.* Disrupted-In-Schizophrenia 1 regulates integration of newly generated neurons in the adult brain. *Cell* **130**, 1146-1158, doi:10.1016/j.cell.2007.07.010 (2007).

73 Iwata, Y. *et al.* Irradiation in adulthood as a new model of schizophrenia. *PloS one* **3**, e2283, doi:10.1371/journal.pone.0002283 (2008).

74 Loganovsky, K. N. & Loganovskaja, T. K. Schizophrenia spectrum disorders in persons exposed to ionizing radiation as a result of the Chernobyl accident. *Schizophrenia bulletin* **26**, 751-773 (2000).

75 Loganovsky, K. N., Volovik, S. V., Manton, K. G., Bazyka, D. A. & Flor-Henry, P. Whether ionizing radiation is a risk factor for schizophrenia spectrum disorders? *The world journal of biological psychiatry : the official journal of the World Federation of Societies of Biological Psychiatry* **6**, 212-230, doi:10.1080/15622970510029876 (2005).

76 Gould, E., Beylin, A., Tanapat, P., Reeves, A. & Shors, T. J. Learning enhances adult neurogenesis in the hippocampal formation. *Nature neuroscience* **2**, 260-265, doi:10.1038/6365 (1999).

77 Leuner, B. *et al.* Learning enhances the survival of new neurons beyond the time when the hippocampus is required for memory. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **24**, 7477-7481, doi:10.1523/JNEUROSCI.0204-04.2004 (2004).

78 Tashiro, A., Sandler, V. M., Toni, N., Zhao, C. & Gage, F. H. NMDA-receptor-mediated, cell-specific integration of new neurons in adult dentate gyrus. *Nature* **442**, 929-933, doi:10.1038/nature05028 (2006).

79 Bruel-Jungerman, E., Davis, S., Rampon, C. & Laroche, S. Long-term potentiation enhances neurogenesis in the adult dentate gyrus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **26**, 5888-5893, doi:10.1523/JNEUROSCI.0782-06.2006 (2006).

80 Zhu, C. *et al.* Isoflurane anesthesia induced persistent, progressive memory impairment, caused a loss of neural stem cells, and reduced neurogenesis in young, but not adult, rodents. *Journal of cerebral blood flow and metabolism : official journal of the International*

Society of Cerebral Blood Flow and Metabolism **30**, 1017-1030, doi:10.1038/jcbfm.2009.274 (2010).

81 Fredriksson, A., Ponten, E., Gordh, T. & Eriksson, P. Neonatal exposure to a combination of N-methyl-D-aspartate and gamma-aminobutyric acid type A receptor anesthetic agents potentiates apoptotic neurodegeneration and persistent behavioral deficits. *Anesthesiology* **107**, 427-436, doi:10.1097/01.anes.0000278892.62305.9c (2007).

82 Slikker, W., Jr. *et al.* Ketamine-induced neuronal cell death in the perinatal rhesus monkey. *Toxicological sciences : an official journal of the Society of Toxicology* **98**, 145-158, doi:10.1093/toxsci/kfm084 (2007).

83 Witcher, M. R., Kirov, S. A. & Harris, K. M. Plasticity of perisynaptic astroglia during synaptogenesis in the mature rat hippocampus. *Glia* **55**, 13-23, doi:10.1002/glia.20415 (2007).

84 Haydon, P. G. & Carmignoto, G. Astrocyte control of synaptic transmission and neurovascular coupling. *Physiological reviews* **86**, 1009-1031, doi:10.1152/physrev.00049.2005 (2006).

85 Rothstein, J. D. *et al.* Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* **16**, 675-686 (1996).

86 Catalani, A. *et al.* Glial fibrillary acidic protein immunoreactive astrocytes in developing rat hippocampus. *Mechanisms of ageing and development* **123**, 481-490 (2002).

87 Nixdorf-Bergweiler, B. E., Albrecht, D. & Heinemann, U. Developmental changes in the number, size, and orientation of GFAP-positive cells in the CA1 region of rat hippocampus. *Glia* **12**, 180-195, doi:10.1002/glia.440120304 (1994).

88 Ullian, E. M., Sapperstein, S. K., Christopherson, K. S. & Barres, B. A. Control of synapse number by glia. *Science* **291**, 657-661, doi:10.1126/science.291.5504.657 (2001).

89 Christopherson, K. S. *et al.* Thrombospondins are astrocyte-secreted proteins that promote CNS synaptogenesis. *Cell* **120**, 421-433, doi:10.1016/j.cell.2004.12.020 (2005).

90 Ge, W. P., Miyawaki, A., Gage, F. H., Jan, Y. N. & Jan, L. Y. Local generation of glia is a major astrocyte source in postnatal cortex. *Nature* **484**, 376-380, doi:10.1038/nature10959 (2012).

91 Nishida, H. & Okabe, S. Direct astrocytic contacts regulate local maturation of dendritic spines. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **27**, 331-340, doi:10.1523/JNEUROSCI.4466-06.2007 (2007).

92 Hirrlinger, J., Hulsmann, S. & Kirchhoff, F. Astroglial processes show spontaneous motility at active synaptic terminals in situ. *The European journal of neuroscience* **20**, 2235-2239, doi:10.1111/j.1460-9568.2004.03689.x (2004).

93 Bernardinelli, Y. *et al.* Activity-dependent structural plasticity of perisynaptic astrocytic domains promotes excitatory synapse stability. *Current biology : CB* **24**, 1679-1688, doi:10.1016/j.cub.2014.06.025 (2014).

94 Haber, M., Zhou, L. & Murai, K. K. Cooperative astrocyte and dendritic spine dynamics at hippocampal excitatory synapses. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **26**, 8881-8891, doi:10.1523/JNEUROSCI.1302-06.2006 (2006).

95 Biederer, T. *et al.* SynCAM, a synaptic adhesion molecule that drives synapse assembly. *Science* **297**, 1525-1531, doi:10.1126/science.1072356 (2002).

96 Fogel, A. I. *et al.* N-glycosylation at the SynCAM (synaptic cell adhesion molecule) immunoglobulin interface modulates synaptic adhesion. *The Journal of biological chemistry* **285**, 34864-34874, doi:10.1074/jbc.M110.120865 (2010).

97 Robbins, E. M. *et al.* SynCAM 1 adhesion dynamically regulates synapse number and impacts plasticity and learning. *Neuron* **68**, 894-906, doi:10.1016/j.neuron.2010.11.003 (2010).

98 Sara, Y. *et al.* Selective capability of SynCAM and neuroligin for functional synapse assembly. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **25**, 260-270, doi:10.1523/JNEUROSCI.3165-04.2005 (2005).

99 Scheiffele, P., Fan, J., Choih, J., Fetter, R. & Serafini, T. Neuroligin expressed in nonneuronal cells triggers presynaptic development in contacting axons. *Cell* **101**, 657-669 (2000).

100 Prange, O., Wong, T. P., Gerrow, K., Wang, Y. T. & El-Husseini, A. A balance between excitatory and inhibitory synapses is controlled by PSD-95 and neuroligin. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 13915-13920, doi:10.1073/pnas.0405939101 (2004).

101 Graf, E. R., Zhang, X., Jin, S. X., Linhoff, M. W. & Craig, A. M. Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. *Cell* **119**, 1013-1026, doi:10.1016/j.cell.2004.11.035 (2004).

102 Zeidan, A. & Ziv, N. E. Neuroligin-1 loss is associated with reduced tenacity of excitatory synapses. *PloS one* **7**, e42314, doi:10.1371/journal.pone.0042314 (2012).

103 Kwon, H. B. *et al.* Neuroligin-1-dependent competition regulates cortical synaptogenesis and synapse number. *Nature neuroscience* **15**, 1667-1674, doi:10.1038/nn.3256 (2012).

104 Chih, B., Afridi, S. K., Clark, L. & Scheiffele, P. Disorder-associated mutations lead to functional inactivation of neuroligins. *Human molecular genetics* **13**, 1471-1477, doi:10.1093/hmg/ddh158 (2004).

105 Chih, B., Engelman, H. & Scheiffele, P. Control of excitatory and inhibitory synapse formation by neuroligins. *Science* **307**, 1324-1328, doi:10.1126/science.1107470 (2005).

106 Chih, B., Gollan, L. & Scheiffele, P. Alternative splicing controls selective trans-synaptic interactions of the neuroligin-neurexin complex. *Neuron* **51**, 171-178, doi:10.1016/j.neuron.2006.06.005 (2006).

107 Galimberti, I. *et al.* Long-term rearrangements of hippocampal mossy fiber terminal connectivity in the adult regulated by experience. *Neuron* **50**, 749-763, doi:10.1016/j.neuron.2006.04.026 (2006).

108 Krzisch, M. *et al.* Propofol anesthesia impairs the maturation and survival of adult-born hippocampal neurons. *Anesthesiology* **118**, 602-610, doi:10.1097/ALN.0b013e3182815948 (2013).

109 Schnell, E., Bensen, A. L., Washburn, E. K. & Westbrook, G. L. Neuroligin-1 overexpression in newborn granule cells in vivo. *PloS one* **7**, e48045, doi:10.1371/journal.pone.0048045 (2012).

110 Sun, G. J. *et al.* Seamless reconstruction of intact adult-born neurons by serial end-block imaging reveals complex axonal guidance and development in the adult hippocampus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **33**, 11400-11411, doi:10.1523/JNEUROSCI.1374-13.2013 (2013).

111 Stagi, M., Fogel, A. I. & Biederer, T. SynCAM 1 participates in axo-dendritic contact assembly and shapes neuronal growth cones. *Proceedings of the National Academy of Sciences of the United States of America* **107**, 7568-7573, doi:10.1073/pnas.0911798107 (2010).

112 Yasuda, M. *et al.* Multiple forms of activity-dependent competition refine hippocampal circuits in vivo. *Neuron* **70**, 1128-1142, doi:10.1016/j.neuron.2011.04.027 (2011).

113 Yuste, R. & Bonhoeffer, T. Genesis of dendritic spines: insights from ultrastructural and imaging studies. *Nature reviews. Neuroscience* **5**, 24-34, doi:10.1038/nrn1300 (2004).

114 Jessberger, S. *et al.* Seizure-associated, aberrant neurogenesis in adult rats characterized with retrovirus-mediated cell labeling. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **27**, 9400-9407, doi:10.1523/JNEUROSCI.2002-07.2007 (2007).

115 Jessberger, S. *et al.* Epigenetic modulation of seizure-induced neurogenesis and cognitive decline. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **27**, 5967-5975, doi:10.1523/JNEUROSCI.0110-07.2007 (2007).

116 Bielefeld, P., van Vliet, E. A., Gorter, J. A., Lucassen, P. J. & Fitzsimons, C. P. Different subsets of newborn granule cells: a possible role in epileptogenesis? *The European journal of neuroscience* **39**, 1-11, doi:10.1111/ejn.12387 (2014).

117 Sauvageot, C. M. & Stiles, C. D. Molecular mechanisms controlling cortical gliogenesis. *Current opinion in neurobiology* **12**, 244-249 (2002).

118 Albertson, T. E., Walby, W. F., Stark, L. G. & Joy, R. M. The effect of propofol on CA1 pyramidal cell excitability and GABAA-mediated inhibition in the rat hippocampal slice. *Life sciences* **58**, 2397-2407 (1996).

119 Yip, G. M. *et al.* A propofol binding site on mammalian GABAA receptors identified by photolabeling. *Nature chemical biology* **9**, 715-720, doi:10.1038/nchembio.1340 (2013).

120 Yamakura, T., Sakimura, K., Shimoji, K. & Mishina, M. Effects of propofol on various AMPA-, kainate- and NMDA-selective glutamate receptor channels expressed in Xenopus oocytes. *Neuroscience letters* **188**, 187-190 (1995).

121 Irifune, M. *et al.* Propofol-induced anesthesia in mice is mediated by gamma-aminobutyric acid-A and excitatory amino acid receptors. *Anesthesia and analgesia* **97**, 424-429, table of contents (2003).

122 Schulte, D. *et al.* Propofol decreases stimulated dopamine release in the rat nucleus accumbens by a mechanism independent of dopamine D2, GABAA and NMDA receptors. *British journal of anaesthesia* **84**, 250-253 (2000).

123 Bianchi, M. T., Botzolakis, E. J., Lagrange, A. H. & Macdonald, R. L. Benzodiazepine modulation of GABA(A) receptor opening frequency depends on activation context: a patch clamp and simulation study. *Epilepsy research* **85**, 212-220, doi:10.1016/j.eplepsyres.2009.03.007 (2009).

124 Lieberwirth, C. & Wang, Z. The social environment and neurogenesis in the adult Mammalian brain. *Frontiers in human neuroscience* **6**, 118, doi:10.3389/fnhum.2012.00118 (2012).

125 Lieberwirth, C., Liu, Y., Jia, X. & Wang, Z. Social isolation impairs adult neurogenesis in the limbic system and alters behaviors in female prairie voles. *Hormones and behavior* **62**, 357-366, doi:10.1016/j.yhbeh.2012.03.005 (2012).

126 Stranahan, A. M., Khalil, D. & Gould, E. Social isolation delays the positive effects of running on adult neurogenesis. *Nature neuroscience* **9**, 526-533, doi:10.1038/nn1668 (2006).

127 Alexander, G. M. *et al.* Remote control of neuronal activity in transgenic mice expressing evolved G protein-coupled receptors. *Neuron* **63**, 27-39, doi:10.1016/j.neuron.2009.06.014 (2009).

128 Armbruster, B. N., Li, X., Pausch, M. H., Herlitze, S. & Roth, B. L. Evolving the lock to fit the key to create a family of G protein-coupled receptors potently activated by an inert ligand. *Proceedings of the National Academy of Sciences of the United States of America* **104**, 5163-5168, doi:10.1073/pnas.0700293104 (2007).

129 van Harten, A. E., Scheeren, T. W. & Absalom, A. R. A review of postoperative cognitive dysfunction and neuroinflammation associated with cardiac surgery and anaesthesia. *Anaesthesia* **67**, 280-293, doi:10.1111/j.1365-2044.2011.07008.x (2012).

130 Bernardinelli, Y. & Chatton, J. Y. Differential effects of glutamate transporter inhibitors on the global electrophysiological response of astrocytes to neuronal stimulation. *Brain research* **1240**, 47-53, doi:10.1016/j.brainres.2008.09.014 (2008).

131 Zhang, J. M. *et al.* ATP released by astrocytes mediates glutamatergic activity-dependent heterosynaptic suppression. *Neuron* **40**, 971-982 (2003).

132 Portera-Cailliau, C., Pan, D. T. & Yuste, R. Activity-regulated dynamic behavior of early dendritic protrusions: evidence for different types of dendritic filopodia. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **23**, 7129-7142 (2003).

133 Allen, N. J. *et al.* Astrocyte glypicans 4 and 6 promote formation of excitatory synapses via GluA1 AMPA receptors. *Nature* **486**, 410-414, doi:10.1038/nature11059 (2012).

134 Panatier, A. *et al.* Glia-derived D-serine controls NMDA receptor activity and synaptic memory. *Cell* **125**, 775-784, doi:10.1016/j.cell.2006.02.051 (2006).

135 Pascual, O. *et al.* Astrocytic purinergic signaling coordinates synaptic networks. *Science* **310**, 113-116, doi:10.1126/science.1116916 (2005).

136 Irons, H. R. *et al.* Three-dimensional neural constructs: a novel platform for neurophysiological investigation. *Journal of neural engineering* **5**, 333-341, doi:10.1088/1741-2560/5/3/006 (2008).

137 Akaneya, Y. *et al.* Ephrin-A5 and EphA5 interaction induces synaptogenesis during early hippocampal development. *PloS one* **5**, e12486, doi:10.1371/journal.pone.0012486 (2010).

138 Carmona, M. A., Murai, K. K., Wang, L., Roberts, A. J. & Pasquale, E. B. Glial ephrin-A3 regulates hippocampal dendritic spine morphology and glutamate transport. *Proceedings of the National Academy of Sciences of the United States of America* **106**, 12524-12529, doi:10.1073/pnas.0903328106 (2009).

139 Henkemeyer, M., Itkis, O. S., Ngo, M., Hickmott, P. W. & Ethell, I. M. Multiple EphB receptor tyrosine kinases shape dendritic spines in the hippocampus. *The Journal of cell biology* **163**, 1313-1326, doi:10.1083/jcb.200306033 (2003).

140 Kayser, M. S., McClelland, A. C., Hughes, E. G. & Dalva, M. B. Intracellular and trans-synaptic regulation of glutamatergic synaptogenesis by EphB receptors. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **26**, 12152-12164, doi:10.1523/JNEUROSCI.3072-06.2006 (2006).

141 Wilkinson, D. G. Multiple roles of EPH receptors and ephrins in neural development. *Nature reviews. Neuroscience* **2**, 155-164, doi:10.1038/35058515 (2001).

142 Murai, K. K., Nguyen, L. N., Irie, F., Yamaguchi, Y. & Pasquale, E. B. Control of hippocampal dendritic spine morphology through ephrin-A3/EphA4 signaling. *Nature neuroscience* **6**, 153-160, doi:10.1038/nn994 (2003).

143 Filosa, A. *et al.* Neuron-glia communication via EphA4/ephrin-A3 modulates LTP through glial glutamate transport. *Nature neuroscience* **12**, 1285-1292, doi:10.1038/nn.2394 (2009).

144 Zhao, S. *et al.* Fluorescent labeling of newborn dentate granule cells in GAD67-GFP transgenic mice: a genetic tool for the study of adult neurogenesis. *PloS one* **5**, doi:10.1371/journal.pone.0012506 (2010).

145 Revest, J. M. *et al.* Adult hippocampal neurogenesis is involved in anxiety-related behaviors. *Molecular psychiatry* **14**, 959-967, doi:10.1038/mp.2009.15 (2009).

146 Sapolsky, R. M. Is impaired neurogenesis relevant to the affective symptoms of depression? *Biological psychiatry* **56**, 137-139, doi:10.1016/j.biopsych.2004.04.012 (2004).

147 Drew, M. R. & Hen, R. Adult hippocampal neurogenesis as target for the treatment of depression. *CNS & neurological disorders drug targets* **6**, 205-218 (2007).

148 Lukkes, J. L., Watt, M. J., Lowry, C. A. & Forster, G. L. Consequences of post-weaning social isolation on anxiety behavior and related neural circuits in rodents. *Frontiers in behavioral neuroscience* **3**, 18, doi:10.3389/neuro.08.018.2009 (2009).

149 Toth, I. & Neumann, I. D. Animal models of social avoidance and social fear. *Cell and tissue research* **354**, 107-118, doi:10.1007/s00441-013-1636-4 (2013).

150 Sahay, A. *et al.* Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. *Nature* **472**, 466-470, doi:10.1038/nature09817 (2011).

151 Burke, S. N. & Barnes, C. A. Neural plasticity in the ageing brain. *Nature reviews. Neuroscience* **7**, 30-40, doi:10.1038/nrn1809 (2006).

152 Kuhn, H. G., Dickinson-Anson, H. & Gage, F. H. Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **16**, 2027-2033 (1996).

153 Haydon, P. G. GLIA: listening and talking to the synapse. *Nature reviews. Neuroscience* **2**, 185-193, doi:10.1038/35058528 (2001).

154 Steib, K., Schaffner, I., Jagasia, R., Ebert, B. & Lie, D. C. Mitochondria modify exercise-induced development of stem cell-derived neurons in the adult brain. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **34**, 6624-6633, doi:10.1523/JNEUROSCI.4972-13.2014 (2014).

155 Petzold, G. C. & Murthy, V. N. Role of astrocytes in neurovascular coupling. *Neuron* **71**, 782-797, doi:10.1016/j.neuron.2011.08.009 (2011).

156 Ji, K., Akgul, G., Wollmuth, L. P. & Tsirka, S. E. Microglia actively regulate the number of functional synapses. *PloS one* **8**, e56293, doi:10.1371/journal.pone.0056293 (2013).

157 Paolicelli, R. C. *et al.* Synaptic pruning by microglia is necessary for normal brain development. *Science* **333**, 1456-1458, doi:10.1126/science.1202529 (2011).

158 Kettenmann, H., Kirchhoff, F. & Verkhratsky, A. Microglia: new roles for the synaptic stripper. *Neuron* **77**, 10-18, doi:10.1016/j.neuron.2012.12.023 (2013).

159 Sierra, A. *et al.* Microglia shape adult hippocampal neurogenesis through apoptosis-coupled phagocytosis. *Cell stem cell* **7**, 483-495, doi:10.1016/j.stem.2010.08.014 (2010).

160 Monje, M. L., Toda, H. & Palmer, T. D. Inflammatory blockade restores adult hippocampal neurogenesis. *Science* **302**, 1760-1765, doi:10.1126/science.1088417 (2003).

161 Waterhouse, E. G. *et al.* BDNF promotes differentiation and maturation of adult-born neurons through GABAergic transmission. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **32**, 14318-14330, doi:10.1523/JNEUROSCI.0709-12.2012 (2012).

162 Sairanen, M., Lucas, G., Ernfors, P., Castren, M. & Castren, E. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. *The Journal of neuroscience : the official journal of the Society for Neuroscience* **25**, 1089-1094, doi:10.1523/JNEUROSCI.3741-04.2005 (2005).

163 Anderson, M. L., Sisti, H. M., Curlik, D. M., 2nd & Shors, T. J. Associative learning increases adult neurogenesis during a critical period. *The European journal of neuroscience* **33**, 175-181, doi:10.1111/j.1460-9568.2010.07486.x (2011).

164 Taupin, P. & Gage, F. H. Adult neurogenesis and neural stem cells of the central nervous system in mammals. *Journal of neuroscience research* **69**, 745-749, doi:10.1002/jnr.10378 (2002).

Articles