Current Topics in Behavioral Neurosciences 15

Catherine Belzung
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Neurogenesis and Neural Plasticity

Springer

Current Topics in Behavioral Neurosciences

Volume 15

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Neurogenesis and Neural Plasticity

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ISSN 1866-3370 ISSN 1866-3389 (electronic)
ISBN 978-3-642-36231-6 ISBN 978-3-642-36232-3 (eBook)
DOI 10.1007/978-3-642-36232-3
Springer Heidelberg New York Dordrecht London

Library of Congress Control Number: 2013936957

Printed on acid-free paper

Springer is part of Springer Science+Business Media (www.springer.com)

Preface

Neuroplasticity is a key feature of adult brain function, enabling adaptation to a continuously changing environment. In the past, it was thought that this process was only significant during the developmental period, making the adult brain relatively inflexible. This idea became hard-wired in the scientific culture, as a dogma, despite evidence from 100 years ago showing that some brain cells are able to undergo mitosis. Proof that these proliferating cells become new neurons required more potent cellular imaging techniques such as confocal microscopy together with the ability to identify cells by double immunolabelling. The same applies to synaptogenesis, as evidence showing that new synapses are constantly being formed and eliminated in the brain required the development of specific novel methodologies. However, behavioral observations, showing that subjects continually learn new information and new procedures throughout their life, strongly suggested a requirement for plasticity and that neuronal changes might underlie such flexibility.

For more than 20 years, it has been recognized that these processes, while decreasing with age, occur in some specific areas of the adult brain, from early adulthood to senescence, and are necessary for behavioral and cognitive flexibility. This plasticity can be morphological, as in the generation of new synapses and new neurons or functional when the strength of synapses changes; as in long-term potentiation or long-term depression. While adult neurogenesis appears to be mainly restricted to the hippocampus and the olfactory system, synaptogenesis and its associated functional changes in plasticity occur in all cortical and subcortical areas. Thus relating these changes to a large number of divergent functions, associated with the brain areas in which this occurs. Synaptogenesis is a rapid process, as formation of new synapses or dendritic spines requires less than two hours. The functional importance of synaptogenesis is closely related to the function of the brain areas in which it takes place, and can thus provide increased flexibility when occurring in frontal or hippocampal areas, or decreased flexibility in the amygdala or some parts of the striatum. Alterations in adult neurogenesis can produce changes in cognition which might participate in the triggering of

pathological conditions such as psychiatric and neurological conditions. Some conditions such as aging or stress induce a decrease in neurogenesis and a remodelling of synaptogenesis (decreased in some regions, and increased in others).

This volume aims to provide a synthetic overview of current findings in neuroplasticity. The first part focuses on the characterization of these processes. While many aspects of memory formation and retrieval are still uncertain, it is now clear that changes in synaptic strength and the recruitment of newly formed neurons to form network units underlies many features of memory. The molecular mechanisms in the formation and regulation of these networks together with the generation of new neurons from stem cells are discussed in chapters by Mariana Carastore, Yan Gu, and Ilias Kazanis and their co authors. The chapter of Gilles Gheusi et al. investigates the characteristics of neurogenesis in the olfactory bulbs, focusing on the behavioral consequences of their functional integration. The chapter by Andrea Gómez-Palacio-Shjetnan and Martha Escobar concerns neurotrophins, particularly Brain-derived Neurotrophic Factor (BDNF) and neurotrophin-3 (NT3), and their role in the stabilization and maturation of already existing synapses, as well as in their ability to generate new synaptic contacts. This is crucial for learning and memory.

The external features of an individual's environment have a major impact on brain plasticity and this is covered in the second part of this book. The chapter by Timothy J. Schoenfeld and Elizabeth Gould provides a new view about the effects of stress and of stress hormones such as glucocorticoids on hippocampal neurogenesis. This chapter attempts to understand the paradoxical finding that certain situations, such as physical exercise or an enriched environment which induce a release of glucocorticoids actually increase neurogenesis in contrast to other stressful situations where these hormones have the opposite effect. The chapter by Michael J. Eckert and Wickliffe C. Abraham is more focused on functional plasticity; it shows how an early transient increase in cell activity might elicit long-term enhancements in cellular and network function necessary for hippocampus-dependent cognition. Subsequent chapters in this part by Peter Wigmore, Carmen Vivar and co-authors, cover the negative impact of chemotherapy on neurogenesis and memory, while physical exercise improves cognition and may in fact be a means to treat chemotherapy-induced memory decline.

The third part of the book describes alterations in brain plasticity occurring in pathological conditions including psychiatric disorders such as depression or addiction and neurological conditions such as neurodegeneration. The chapter by Francis Bambico and Catherine Belzung describes the synaptic reorganization and hippocampal neurogenesis in relation to depression and to the effects of antidepressants. It proposes a comprehensive view regarding the dynamic of these changes, suggesting that antidepressant therapy first increases synaptogenesis in frontal areas, which facilitates the initiation of recovery, and then stimulates neurogenesis, which enables the therapeutic effects to become long lasting, preventing recurrence. The chapter by Juan Canales provides evidence suggesting that hippocampal neurogenesis might be involved in the emergence and maintenance

of addictive behavior. Indeed, several components of the downward spiraling loop that characterizes addiction, including elevated sensitivity to drug-induced reward and reinforcement, enhanced neurohormonal responsiveness, emergence of a negative affective state, memory impairment, and inflexible behavior are all related to neurogenesis. Alzheimer's disease with its associated decline in cognition is a major medical concern with an increasingly aging population. The use of transgenic animal models which mimic aspects of the disease and can now be used to test potential treatments is discussed by Michael Marlatt and co-authors.

Finally, Aging and Repair are crucial processes related to neuroplasticity. The chapter by Sebastian Couillard-Despres reports studies showing a decline of hippocampal neurogenesis associated with normal aging. However, these changes probably do not underlie the decrease in cognitive function seen in older subjects, as current studies suggest a distinct role of hippocampal neurogenesis in young versus adult and old brain. Stem cell therapy offers the tantalizing prospect of repairing the damaged or degenerating brain. Rebecca Trueman and co-authors discuss the use of endogenous and transplanted stem cells to repair Parkinson's and Huntington's disease and stroke.

We have only started to understand the nature and importance of plasticity to the functioning of the brain. The ability to change and build new circuits in response to both internal and external stimuli makes the brain uniquely able to cope with the demands imposed upon it. This volume discusses recent work ranging from theoretical aspects of cognition, to the detailed cellular and molecular biology mechanisms which underlie the functional changes the brain can make. An understanding of these is now opening up avenues to influence these events in the treatment of a wide range of neurological and psychiatric conditions. These are indeed exciting times to be working on brain plasticity and neurogenesis and the authors gathered together here convey much of this excitement in what is becoming a rapidly expanding field.

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Part I
Characterization

Neurogenesis in the Adult Mammalian Brain: How Much Do We Need, How Much Do We Have?

Ilias Kazanis

Abstract The last two decades cytogenic processes (both neurogenic and gliogenic) driven by neural stem cells surviving within the adult mammalian brain have been extensively investigated. It is now well established that within at least two cytogenic niches, the subependymal zone of the lateral ventricles and the subgranular zone in the dentate gyrus, new neurons are born everyday with a fraction of them being finally incorporated into established neuronal networks in the olfactory bulb and the hippocampus, respectively. But how significant is adult neurogenesis in the context of the mature brain and what are the possibilities that these niches can contribute significantly in tissue repair after degenerative insults, or in the restoration of normal hippocampal function in the context of mental and cognitive disorders? Here, we summarise the available data on the normal behaviour of adult neural stem cells in the young and the aged brain and on their response to degeneration. Focus will be given, whenever possible, to numbers: how many stem cells survive in the adult brain, how many cells they can generate and at what ratios do they produce neurons and glia?

Keywords Neurogenesis · Gliogenesis · Neural stem cells · Progenitors · Subependymal zone/subventricular zone · Subgranural zone · Regeneration · Memory · Hippocampus

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Curr Topics Behav Neurosci (2013) 15: 3–29
DOI: 10.1007/7854_2012_227

Published Online: 14 September 2012

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1 Numbers Matter and Two Cautions

During the long process of evolution the brain has—on average—increased in size, but most importantly in complexity of structure (emergence of neocortex and subsequently of gyrencephalia) and of connectivity, with the latter—among mammals—being correlated to the emergence of more complex patterns of behaviour (Herculano-Houzel 2009). The striking increase in the size of the brain has been underlined by the appearance of new neurogenic progenitor populations, such that the "ancient" neuroepithelial cells that (early in evolution and in embryogenesis) form the primitive embryonic neural tube are transformed to radial glial cells when the tube becomes thicker and these are subsequently complimented by subventricular zone progenitors (for the neocortex to appear) and by outer subventricular zone progenitors (for gyrencephalia to appear) (Fietz et al. 2010; Stancik et al. 2010). Interestingly, the emergence of larger and more complicated brains has been accompanied by a marked decrease in the number of neurogenic progenitors surviving within the adult, mature tissue. Not only certain adult neurogenic areas (or niches) disappeared during evolution but also the absolute numbers of neuronal progenitors per area has decreased (Ferretti 2011; Kazanis and ffrench-Constant 2012). This has resulted in a significant weakening of the neuroregenerative capacity of the adult brain; thus, to a higher susceptibility to injury. But why has that happened? Intuitively, we believe that the increased complexity of the brain, with the emergence of very specialised neuronal types that are interconnected via numerous axonal networks, is not compatible with efficient cell-replacement machineries. The additional observation that cell replacement of glial cells—cells considered to be only supportive to neurons and more homogeneous in morphology and function—has won the battle of survival during evolution turned this intuitive belief to a "dogma" that dominated developmental neurobiology to such a degree that for many decades the first indications of persistent neurogenic activity in the adult rodent brain by Altman (1969) were dismissed. On the other hand, why would a neuronal progenitor, located within a specialised and protective microenvironment in the brain, be worried about what happens to its progeny? How can an inefficient cell-replacement process impact directly on the progenitor cell, since the vast majority of stem cell progeny

normally die before reaching maturity and before being incorporated in existing networks even in the adult neurogenic systems that survived the forces of evolution (Morshead and van der Kooy 1992; Lu et al. 2011)? A possible explanation could be that the enlargement of the brain required more space for mature tissue, leading to a gradual reduction in the size and number of niches, especially since this did not incur any serious functional defects. Another explanation could be sought in energy efficiency pressures, with brains spending less energy for maintaining a futile neurogenic process being positively selected. Finally, recently published data indicate that the cell cycle kinetics of progenitors depend on real time, rather than on developmental time (i.e. a progenitor can keep dividing for a certain number of days/weeks irrespective of whether this means old age in a rodent and infancy in humans) (Amrein et al. 2011). This—among many other possibilities—indicates that the decrease in the numbers of surviving progenitors is a function of time, with organisms with long life duration spending most of their adult lives with small pools of progenitors.

Nevertheless, one of the most important messages stemming from the discovery of persistent neurogenic activity in the adult mammalian brain is that replacement of neurons is not a process completely lost during evolution; and this has great implications in regenerative medicine. It is much easier to exogenously influence an existing cellular process rather than to attempt to re-introduce it after it has vanished. In that context, the discovery that neurogenesis has a direct role in the function of the hippocampus, involving memory and learning (Deng et al. 2010), added a new promising experimental target in the field of mental health disorders and of cognitive biology. Importantly, though, we should not forget that in regenerative and rehabilitation medicine numbers matter: not only the numbers of progenitors surviving in the adult brain, but also the numbers of cells that are affected during degeneration or disease. In this review, I will attempt to summarise the available information on persistent neurogenesis in the adult brain with a focus given, whenever possible, to numbers. Most of the evidence stems from experiments carried out in animals, especially rodents, but whenever appropriate I will discuss findings from the primate (including the human) brain.

Before proceeding further, though, two cautions have to be stated regarding "adult neurogenesis". The first one has to do with the definition of the term "adult". One possible way to define adulthood in the context of a tissue is as "the stage in which the tissue has reached a stable anatomical structure, with no further addition or elimination of subdivisions and which can only be altered by degenerative processes (injury or aging)". In that sense, the brain—or at least some specific structures such as the corpus callosum (Sturrock 1980)—might be reaching adulthood much later than generally considered in rodents and much earlier in humans. In the same direction, recent evidence from the human and rodent brain suggest that the gradual decrease in postnatal neurogenic activity might not be a degenerative process of the adult brain but rather the end of a "prolonged developmental process" with the system in rodents reaching the adult steady state at around 6 months post natally (Ben Abdallah et al. 2010; Knoth et al. 2010; Amrein et al. 2011). In that case, a large volume of experimental work in

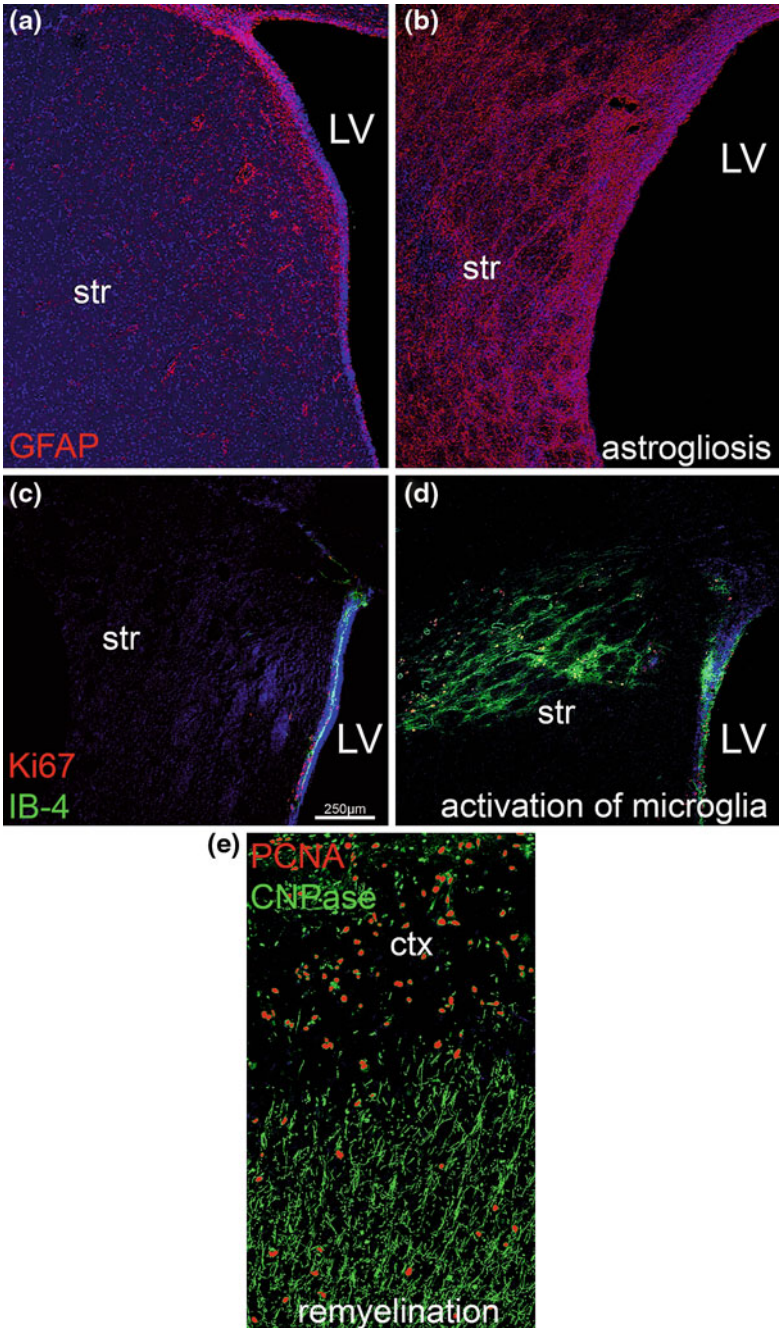
"adult neurogenesis" in the rodents has been performed in a non-adult neurogenic system. Moreover, if the numbers of persisting progenitors are a function of time, then an additional caution has to be made regarding the extrapolation of experimental results from adult rodents (e.g. 3 month old) to the adult human brain (e.g. 30 years old) (Amrein et al. 2011; Sanai et al. 2011).

The second caution has to do with the fact that experiments in the laboratory are performed in animals kept in captivity and in very controlled, stereotypic and stable conditions. Recent data suggest that in mice captured in the wild neurogenesis in the hippocampus is not directly dependent on exercise, as has been shown in laboratory-kept mice (Klaus and Amrein 2012; Klaus et al. 2012). A possible explanation for this discrepancy could be that in experimental conditions the levels of homeostatic neurogenesis are low due to the minimal exposure of animals to stimuli. This would therefore mean that it is "relatively easy" to induce increases in neurogenesis in the hypo-active laboratory animals. Other studies have also showed that specific features of progenitor behaviour (such as cell cycle kinetics) show a rather genetic regulation, while others (such as the rate of differentiation and the rate of survival) are more influenced by the environment (Amrein et al. 2011; Roth et al. 2012). In that sense, the caution regards the extrapolation of results regarding the effects of the administration of chemical substances or of the manipulation of the environment in the levels of neurogenesis in animals kept in captivity to animals living in the wild, such as humans (Hauser et al. 2009).

2 Cellular Plasticity in the Adult Brain; When New Cells are Required?

2.1 Gliogenesis in the Parenchyma of the Brain

Although the brain is widely perceived to be a structurally non-plastic organ, apart from the micro-level of the synapse and of the dendrite, the truth is that whenever appropriately stimulated, brain tissue can host robust proliferative and migratory events. If an area is injured by mechanical forces, cytotoxic substances or hypoxia, the widely spread astrocytes react immediately. They change their morphology by increasing in size and extending processes, they proliferate and they also migrate at the site of lesion. This is called astrogliosis (Fig. 1a, b) and in many cases results in the formation of permanent scars in the tissue (Fawcett and Asher 1999). In exactly the same instances another pool of cells becomes equally activated: microglial cells (Fig. 1c, d). Microglia comprise the innate immune system of the brain and in many cases their response is complimented by blood-borne macrophages that invade the tissue, often through the broken blood–brain barrier (Perry et al. 2010). More impressively, if the insulating and protective myelin sheaths that cover neuronal axons are destroyed due to chemotoxic or autoimmune reasons (a


(a)
LV
str
GFAP
(b)
LV
str
astrogliosis
(c)
str
LV
Ki67
IB-4
250µm
(d)
str
LV
activation of microglia
(e)
PCNA
CNPase
ctx
remyelination

◀ **Fig. 1** Gliogenesis in the brain parenchyma. (Panels **a**, **b**) Microphotographs of adult rat brain tissue immunostained for glial fibrilary acidic protein (GFAP, in *red*), a marker of astrocytes. Numbers of astrocytes increase significantly in response to degeneration, in this case after an experimental model simulating stroke, a phenomenon called astrogliosis. (Panels **c**, **d**) Microphotographs of adult rat brain immunostained for isolectin B4 (IB-4, in *green*) a marker of microglial cells and Ki67 (in *red*) a marker of proliferating cells. A degenerative insult, in this case an experimental model simulating stroke, induces a massive proliferative response of microglial cells. (Panel **e**) Microphotograph of adult mouse brain immunostained for CNPase (in *green*) a marker of cells of the oligodendroglial cell lineage and PCNA (in *red*) a marker of proliferating cells. A demyelinating insult, in this case the focal infusion of lysolecithin (1 %) in the cortex, leads to the destruction of myelin sheaths (note the absence of CNPase positive processes), and to the mitotic activation of oligodendrocyte progenitors that will regenerate lost oligodendrocytes (remyelination). (*ctx* cortex, *LV* lateral ventricle, *str* striatum)

process called demyelination), then another pool of cells, the oligodendrocyte progenitors, are recruited: they proliferate (Fig. 1e), migrate and successfully regenerate myelin sheaths (remyelination) (Franklin and ffrench-Constant 2008). Importantly, in recent years evidence has appeared indicating that these processes, that were considered to be relevant only to the world of glial cells, might hide a more dynamic capacity. Astrocytes activated by a focal cortical injury were shown to be able to act as multipotent progenitors when cultured in vitro (Buffo et al. 2008), while oligodendrocyte progenitors responding to focal demyelination were found to generate cells of the peripheral nervous system (schwann cells) and neurons in vivo (Guo et al. 2010; Zawadzka et al. 2010).

2.2 Neurogenesis/Gliogenesis in Cytogenic Niches of the Brain

Surprisingly, and in contrast to the above-mentioned glial cell-replacement processes that operate in very low levels in the homeostatic adult brain and show a robust response after degeneration, neuron cell-replacement processes show a higher daily level of activity during homeostasis and serve specific functional needs. Adult neurogenesis is almost exclusively driven by pools of adult neural stem cells that survive in two specialized microenvironments in the adult brain: in the subependymal zone (SEZ/often called subventricular zone) of the lateral walls of the lateral ventricles and in the subgranular zone (SGZ) of the dentate gyrus in the hippocampus (Doetsch et al. 1997; Seri et al. 2004) (Fig. 2). The same cytogenic niches also generate glial cells (astrocytes and oligodendrocytes), though in much lower frequencies.

2.2.1 The Architecture of Cytogenic Niches

The process of cell generation is similar in the two neurogenic niches of the adult rodent brain; therefore, the main cell types located therein are also similar. NSCs of astroglial morphology that remain relatively quiescent generate precursors of

neuronal commitment that are called neuroblasts and express doublecortin and the polysialiated form of NCAM, via a cell amplification step (Fig. 2). In the SEZ, transit amplifying precursors undergo many symmetric self-renewing divisions before producing committed progenitors (Morshead et al. 1998; Doetsch et al. 1999) and express the transcription factor Mash1 (Parras et al. 2004) and the receptor for epidermal growth factor (Doetsch et al. 2002). Apart from neuron-committed progenitors few recent reports have demonstrated the generation of oligodendrocyte progenitors from the SEZ that are subsequently migrating to the corpus callosum (Jackson et al. 2006; Menn et al. 2006; Etxeberria et al. 2010; Jablonska et al. 2010). Although the process of lineage commitment remains largely unexplored the available evidence indicates both the existence of separate populations of transit amplifying progenitors (with either neuronal or glial commitment, expressing transcription factors Pax6 and Olig2, respectively) (Hack et al. 2005) and that cells of neuronal fate retain the ability to transdifferentiate into oligodendroglial progenitors upon gliogenic stimulation (Jablonska et al. 2010). Approximately 15,000 cells are born every day in each SEZ (Lois and Alvarez-Buylla 1994) and the vast majority of these are neuroblasts. The average ratio of oligodendrogenesis versus neurogenesis has been estimated to 1:20, with approximately the same number of oligodendrocytes being generated in all rostrocaudal levels of the SEZ, but neurogenesis being markedly decreased caudally (Menn et al. 2006). In the SGZ, the volume of cells generated is smaller than that of the SEZ with estimates varying from 9,000 cells (Cameron and McKay 2001) to 4,000 cells per SGZ per day (Rao and Shetty 2004). In the hippocampal niche, the amplification process is limited to 1–2 divisions and transit amplifying progenitors express doublecortin, similar to neuroblasts and immature neurons (Seri et al. 2004), although there is evidence for Mash1 and Tbr2 expression (Yoshihara et al. 2007; Hodge et al. 2008).

Another common structural feature of the two niches is the close distance between proliferating neural precursors and the vasculature (Palmer et al. 2000; Mirzadeh et al. 2008; Shen et al. 2008; Tavazoie et al. 2008). The SEZ neurogenic niche—which has been more extensively described—is preferentially rich in blood vessels as compared to other periventricular domains (Kazanis et al. 2010) with precursors contacting blood vessels in microdomains void of astrocytic endfeet and pericytes (Tavazoie et al. 2008). Regarding the SGZ, in mice subjected to exercise increased neurogenesis was correlated with an increase in vascularisation (Clark et al. 2009). A structural characteristic that is specific for the SEZ is the proximity to the lateral ventricle, from which it is separated by the monolayer of ependymal cells. The importance of ependymal cells and of the regulated communication with the cerebrospinal fluid (that fills the ventricles) in the regulation of neurogenesis is underlined by several findings. Ependymal cells express regulating factors, such as several bone morphogenetic proteins (Colak et al. 2008; Gajera et al. 2010) and pigment epithelium-derived growth factor (Ramirez-Castillejo et al. 2006), while their cilia create a gradient of factors at the ventricular side that instructs the direction of migration to neuroblasts (Sawamoto et al. 2006). Moreover, ependymal cells create specific pinwheel structures in the ventricular

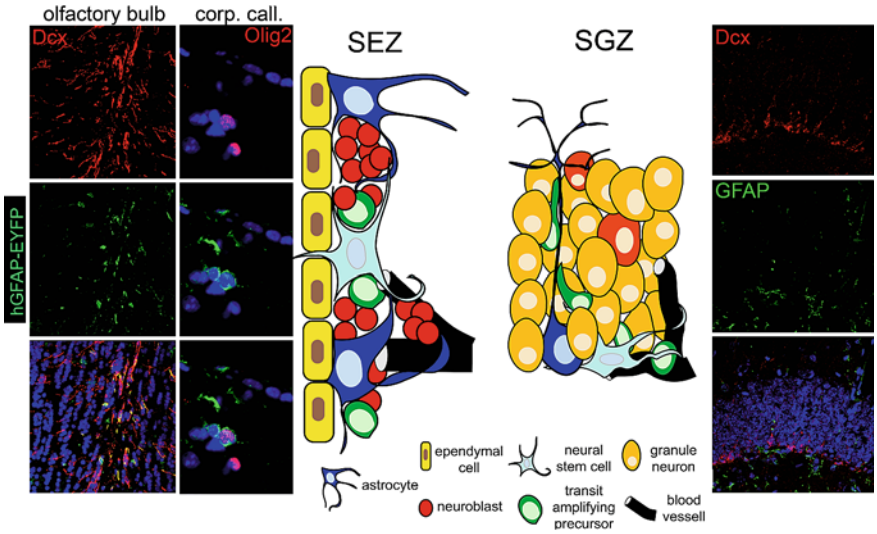


Fig. 2 The cytogenic niches of the adult mammalian brain. Graphic illustration of the cytoarchitecture of the subependymal zone (SEZ/*centtre left*) and the subgranular zone (SGZ/*centre right*) neurogenic niches. Note the common features: adult neural stem cells are of astroglial morphology (*light blue* cells), neurogenesis occurs through an intermediate precursor stage (*green* cells) leading to the generation of immature neurons (*red* cells) and is closely related to the presence of blood vessels (in *black*). Note also the ependymal cell layer that forms the wall of the lateral ventricle at the side of the SEZ and the process of the NSC that intercalates among ependymal cells and contacts the content of the ventricle. In the panels at the *left* side are shown microphotographs of adult mouse brain tissue immunostained for EYFP expressed via the human promoter of GFAP (in *green*, marking astrocytes—including adult NSCs—and their progeny) and either doublecortin (Dcx/in *red*, marking new neurons) or Olig2 (in *red*, marking oligodendrocyte progenitors). Double positive cells (*yellow*) within the olfactory bulbs are SEZ-generated new neurons and within the corpus callosum are SEZ-generated oligodendrocyte progenitors. In the panels, at the *right* side are shown microphotographs of adult mouse brain tissue immunostained for GFAP (in *green*, marking astrocytes) and Dcx (in *red*, marking new neurons). Note the coexistence of astrocytes (some of which are NSCs) and Dcx-positive cells within the thin SGZ at the base of the dentate gyrus. (This figure is an adaptation from Kazanis et al. 2008 published under the Creative Commons Attribution 3.0 Unported License http://creativecommons.org/licenses/by/3.0/)

wall, allowing the regulated intercalation of monociliated processes extended by astrocytes (potentially the NSCs) that contact the content of the ventricles (Doetsch et al. 2002; Mirzadeh et al. 2008) (Fig. 2). Interestingly, such cilium-bearing processes have also been identified on SGZ stem cells (that are not positioned near the ventricle) and are important for adult neurogenesis mainly through regulation of sonic hedgehog signalling (Han et al. 2008). Finally, when comparing the neurogenic and non-neurogenic parts of the ventricular walls two more—possibly important—features can be observed: first, the neurogenic side is rich in myelinated axons (Fig. 3) and second, at the non-neurogenic side an astrocytic layer appears below the ependymal cell layer as if insulating the subependymal area

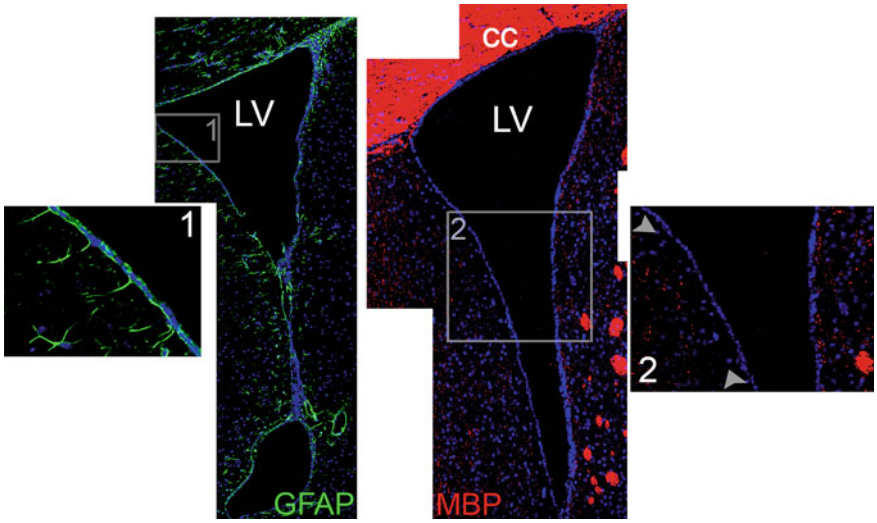


Fig. 3 Structural hallmarks of the SEZ. Microphotographs of adult mouse brain tissue, at the area around the lateral ventricle (LV) immunostained either for GFAP (*left* panels, in *green*, marking astrocytes) or for myelin basic protein (MBP/*right* panels, in *red*, marking myelin sheaths surrounding axons). Note that the neurogenic side (the SEZ niche) is located at the lateral wall of the lateral ventricle (here at the *right* walls of the LV). Also, note the astrocytic layer that is found next to the ventricular wall at the non-neurogenic side (inset 1) and the high density of myelinated axons found adjacent to the ventricular wall only at the neurogenic side (inset 2, with *arrowheads* indicating the axon-poor non-neurogenic side of the LV). (*cc* corpus callosum. The figure is an adaptation from Kazanis et al. 2010)

from the content of the ventricle (Kazanis et al. 2010) (Fig. 3). Another major element in the architecture of the niche is the extracellular matrix. The SEZ, more similar to the embryonic CNS than to mature neural tissue, is rich in laminins, tenascin-C and sulphated proteoglycans (Kazanis et al. 2007, 2010; Akita et al. 2008) with fractones (extensions of the blood vessel basal lamina) contacting almost all the different cellular elements of the niche (Mercier et al. 2002). Various components of this matrix have been shown to be important for the post-natal formation of the niche (Peretto et al. 2005; Kazanis et al. 2007), and the modulation of growth factor activity (von Holst et al. 2006; Kerever et al. 2007; Sirko et al. 2007). Importantly, the interaction between extracellular matrix molecules and their receptors on cells, such as the laminin-integrin interactions, are crucial for regulating the proliferation of progenitors (Shen et al. 2008) and possibly the activation of NSCs (Kazanis et al. 2010).

In addition to cells of the adult NSC lineage and blood vessels, experimental work has also revealed the presence of cells of the innate (in homeostasis) and the blood-borne (after injury) immune system within cytogenic niches in the brain. These cells have been shown to exert important regulatory roles, in vitro (Walton et al. 2006; Thored et al. 2009) but data from in vivo experiments still remain inconclusive. For example, after stroke, the activation of microglial cells can be

both pro-neurogenic (Walton et al. 2006) and anti-neurogenic (Hoehn et al. 2005), while a recent study failed to find any significant impact caused by their ablation (Heldmann et al. 2011). An interesting finding has been generated from animal models of Huntington's disease. Increased neurogenesis was observed only in animal models in which the disease was mimicked by the use of cytotoxic substances, inducing inflammatory reactions, while no change was observed in transgenic models, in which cell loss is minimal (Phillips et al. 2005). In addition, recent experimental work in an animal model of demyelination indicated impaired proliferation caused by the inflammatory microenvironment (Pluchino et al. 2008), while examination of tissue from patients suffering from multiple sclerosis indicated sustained activation of the SEZ within the inflammatory microenvironment created by the disease (Nait-Oumesmar et al. 2007). Nevertheless, locally residing microglial cells are likely to be important in the accurate buffering of cell numbers and local migratory events, as they participate in the removal of dying progenitors (Sierra et al. 2010), surprisingly aided by neuronal progenitors (Lu et al. 2011). Furthermore, niche microglia were shown to have distinct properties from those residing outside, such as the ability for indefinite expansion in vitro (Marshall et al. 2008).

The above presented description of the cytoarchitecture of the niches refers to the rodent brain (most data are derived from experiments in the mouse but they largely apply to the rat). Recent ultrastructural and immunohistochemical analyses of the human and non-human primate brains have revealed the existence of similar neurogenic areas at the SEZ of the lateral walls of the lateral ventricles (Jackson et al. 2006; Fancy et al. 2009) and in the hippocampus (Eriksson et al. 1998). The main differences between the rodent and the human SEZ are: the existence of a hypocellular (gap) area underneath the ependymal cell layer that is followed—deeper in the tissue—by an astrocyte-rich ribbon-like zone, the existence of displaced ependymal cells, the absence of chains of migrating neuroblasts and the absence of transit amplifying precursors (Sanai et al. 2004; Quinones-Hinojosa et al. 2006; Wang et al. 2011). Notably, the human SEZ acquires this distinct, mature architecture as soon as cytogenic activity is drastically reduced, at around 18 months after birth (Sanai et al. 2011). On the other hand, NSCs are of similar astroglial morphology—often in contact with the content of the ventricles—and clusters of neuroblasts are found within the hypocellular zone (Jackson et al. 2006; Fancy et al. 2009). In the Macaque brain, the structure of the SEZ neurogenic niche is similar to that of the human brain, albeit chains of migrating neuroblasts are observed similarly to the rodent SEZ (Jackson et al. 2006).

2.2.2 Focusing on Adult NSCs: How Many—How Plastic?

Two of the factors that determine the regenerative capacity of adult cytogenic niches are the numbers of available NSCs and their differentiation potential. In the absence of an absolute marker for adult NSCs, it is very difficult to estimate their numbers in the adult brain. Three such attempts have been published, all focusing

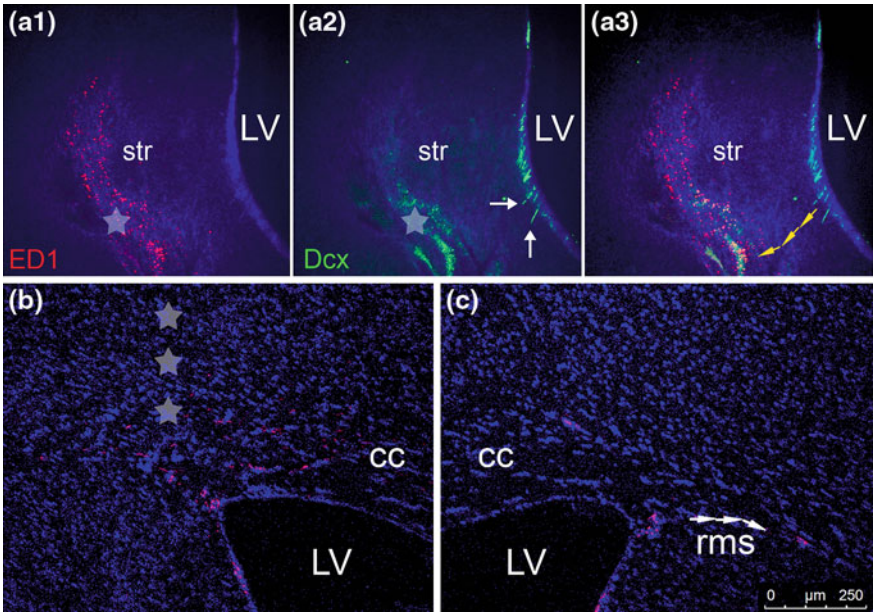


Fig. 4 Activation of the SEZ after degeneration. (Panel **a**) Microphotographs of adult rat brain tissue immunostained for ED1 (in *red*, marking activated macrophages) and Dcx (in *green*, marking newborn neurons). The tissue is taken from a rat subjected to a focal ischaemic insult (unilateral middle cerebral artery occlusion for 1 h). Note that neurons generated in the SEZ form chains (*white arrows*) with a direction towards the core of the lesion (*asterisk*) which is rich in activated macrophages and immature neurons. *Yellow* arrows indicate the possible route of SEZ-generated cells towards the lesion. (Panels **b**, **c**) Microphotographs of adult mouse brain tissue immunostained for Dcx (in *red*, marking neuronal progenitors). Note that although the normal migratory route of SEZ-born progenitors is the rostral migratory stream (rms/indicated with *white arrows* in **c**), which drives them to the olfactory bulb, after a focal demyelinating insult (induced by 1 % lysolecithin, the stars mark the site of demyelination) progenitors are diverted towards the lesion (in **b**). (*cc* corpus callosum, *LV* lateral ventricle, *str* striatum)

in the SEZ and producing a range of values. Two of the studies are more comparable because they are based on the analysis of the regenerating niche, i.e. the NSC-driven repopulation of the niche after the exogenously induced ablation of their downstream daughter cells (transit amplifying progenitors and committed progenitors are actively dividing cells, thus, very sensitive to cytostatic drugs such as AraC or to tritiated thymidine). According to these studies approximately 600 (Morshead et al. 1998), or 300 (Kazanis and ffrench-Constant 2012) NSCs reside within each SEZ. The third study (Golmohammadi et al. 2008) used a combination of in vitro colony-formation assays and in vivo labelling-retention experiments and estimated a much lower number of potential NSCs per SEZ (approximately 50), that is closer to the number of active NSCs at any random time estimated by Kazanis and ffrench-Constant (2012). Interestingly, when the mouse and rat SEZs were compared, the latter was found to contain approximately three times more

NSCs, although its volume was five times bigger (Kazanis and ffrench-Constant 2012). Moreover, it was shown that the size of the neurogenic area was determined by the scale of the whole brain, while the number of NSCs was limited by the number of ependymal cells. This suggests that during evolution the enlargement of the brain (leading to a higher demand for cell-replacement events in the case of degeneration) is not isometrically followed by the enlargement of the NSC pool. This could potentially underline the emergence of the gap zone in the even larger primate brain although more comparative analyses have to be performed in order to test this hypothesis. Notably, both adult brain cytogenic niches are not populated by NSCs in their full (volumetric) capacity. Detailed analyses of the SEZ have revealed areas of high and low density of astrocytic endfeet projecting in the ventricle (interpreted as areas of high and low abundance of NSCs, respectively) (Mirzadeh et al. 2008) and similar results have been obtained by investigating the presence of NSCs in serial coronal vibratome-cut sections (Golmohammadi et al. 2008). In the rat hippocampus, quiescent domains have also been identified within the SGZ of the upper blade of the dorsal dentate gyrus (Gil-Mohapel et al. 2010). Nevertheless, the absolute number of NSCs is only one of the parameters potentially affecting the cytogenic capacity of adult brain niches. Another parameter is the ability of these NSCs to become activated when necessary. Again by investigating the regenerating SEZ, it was estimated that only the mitotic activation of almost the whole NSC population can explain the fast repopulation of the transit amplifying progenitor pool (Kazanis et al. 2007), but only limited data exist directly showing activation of NSCs after degeneration (Zhang et al. 2004).

Adult NSCs retain the cardinal property of all stem cells that is the capacity for inexhaustible self-renewal for the duration of the life span of the organism (Ahlenius et al. 2009). They also retain the multipotentiality of embryonic NSCs, as indicated by their ability to generate—in vivo and in vitro—the three main CNS cell types: neurons, astrocytes and oligodendrocytes (Suh et al. 2007; Jessberger et al. 2008; Scott et al. 2010) (Fig. 5). Amazingly, isolated adult NSCs not only can act as embryonic NSCs when re-introduced in a host neural tube (Neumeister et al. 2009) but can also contribute to all germ layers in chimeric chick and mouse embryos (Clarke et al. 2000). Additional experimental studies showed that SEZ-derived NSCs can differentiate into cells of the hematopoietic lineage when transplanted in the bone marrow of irradiated mice (Bjornson et al. 1999) as well as into muscle cells both in vitro and in vivo (Galli et al. 2000; Rietze et al. 2001). The differentiation potential of SGZ-derived NSCs has not been directly explored, although transplantation experiments have demonstrated that SGZ progenitors can behave similar to SEZ progenitors when grafted in this neurogenic system, suggesting that NSCs of the two distinct niches might be fundamentally analogous (Suhonen et al. 1996).

The differentiation repertoire of adult NSCs is normally restricted, although it is altered in abnormal conditions (such as after injury, or genetic and chemical manipulation) as will be discussed later in this review. NSCs of the SEZ give rise mainly to GABAergic periglomerular and granule cell interneurons (Doetsch et al. 2002) and possibly interneurons of the external plexiform layer (Yang 2008) and

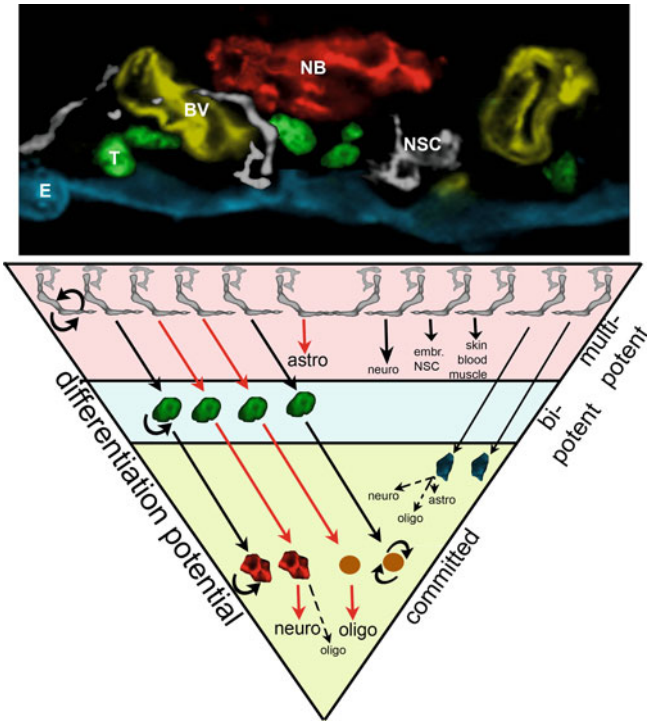


Fig. 5 Differentiation potential of NSCs and of their progeny. (*Top* panel) A collage of microphotographs illustarting the main cellular elements of the cytogenic niche of the SEZ. Ependymal cells (E, *blue*) form the wall of the ventricle (which is at the bottom of the image), adjacent are found the astrocyte-like NSCs (*white*), the transit amplifying progenitors (T, *green*), the neuroblasts (NB, *red*) and multiple blood vessels (BV, *yellow*). (*Lower* panel) Illustration depicting the known differentiation potential of the different cells of the adult NSC lineage (following the colours of the *top* panel). Note that the only cell type not shown in the *top* panel is the SEZ-generated oligodendrocyte progenitor (shown as a *brown circle* in the *lower* panel) that has not been clearly identified so far. The differentiation potential of progenitors follows the shape of a pyramid, with NSCs being at the base (thus exhibiting the widest differentiation potential). The *red* arrows indicate the normal routes of generation of the main three cell types of the central nervous system (neurons, astrocytes, oligodendrocytes) from adult NSCs. Interrupted lines indicate differentiation processes that occur only after degeneration

glutamatergic juxtaglomerular neurons (Colak et al. 2008). The limited plasticity of SEZ NSCs is even more dramatically revealed by the fact that they generate only specific subpopulations of periglomerular cells: those expressing calretinin and tyrosine hydroxylase (Peretto et al. 2004). The other major cell types homeostatically generated by SEZ progenitors are oligodendrocyte precursors and oligodendrocytes (Jackson et al. 2006; Menn et al. 2006; Etxeberria et al. 2010; Jablonska et al. 2010) that migrate short distances and then become stationary within the corpus callosum. SEZ progenitors have also been reported to generate astrocytes (Chmielnicki et al. 2004). However, most of the published cell fate

experiments have not identified SEZ-derived astrocytes outside the niche (Merkle et al. 2007; Havrda et al. 2008; Scott et al. 2010); thus, the level of SEZ astrogliogenesis remains controversial. No data exist regarding the generation of microglial cells from adult NSCs. The plasticity potential of SGZ progenitors is also normally limited; they generate almost exclusively granule neurons, very low numbers of astrocytes (less than 10 % of generated cells) (Suh et al. 2007) and extremely low numbers of early oligodendrocyte progenitors (approximately 3 %) that do not mature efficiently (Jessberger et al. 2008).

2.2.3 Neurogenesis in Homeostasis and the Effects of Ageing

In rodents, the immature neurons generated in the SEZ migrate a long distance in order to reach the olfactory bulb (Lois and Alvarez-Buylla 1994) where they differentiate mainly in interneurons (Fig. 2). Augmenting evidence indicates that the addition of these newborn neurons is essential for odour recognition (Doetsch et al. 2002; Breton-Provencher et al. 2009; Mouret et al. 2009). What still remains unclear is whether these cells replace old neurons (having been generated during embryonic development) or only other neurons previously born in the SEZ (Lemasson et al. 2005; Ninkovic et al. 2007). To have a sense of magnitude, approximately 170,000 cells arrive in the OB from the SEZ in a period of 10 days and 40% of these survive for more than 3 months (Winner et al. 2002). Recent experimental work has shown that the newly incorporated cells behave differently to the old neurons, by for example being more sensitive to plasticity with the expression of long-term potentiation (LTP) (Nissant et al. 2009) and are necessary for short-term olfactory memory (Breton-Provencher et al. 2009). Notably, proper olfactory functionality depends not only to the arrival and integration of new cells, but also to the efficient removal of older cells, as it is disturbed upon inhibition of cell death (Mouret et al. 2009). The NSCs that reside in the SGZ generate new granule neurons; thus, progenitors migrate only short distances (Fig. 2) from the niche until their final destination. As in the olfactory bulb, newly born neurons behave differently from already established “old” cells. Importantly, it takes a few weeks for SGZ-generated neurons to mature; initially, they are not responsive to neuronal activity (Snyder et al. 2009) and gradually start to receive only GABAergic inputs from local interneurons (Esposito et al. 2005; Ge et al. 2006). Subsequently—similar to what happens during development—this GABAergic input becomes inhibitory and glutamatergic input starts to appear. Although these adult-born neurons gradually become morphologically identical to older cells, they exhibit lower threshold for the induction of LTP, thus are more plastic (Ge et al. 2006, 2007) and seem to be more responsive to stimuli generated by animal’s experiences (Deng et al. 2010). Many computational models have indicated that the integration of new neurons is compatible—and even necessary—for the efficient functioning of the dentate gurus and the hippocampus (Kempermann et al. 2004); however, the issue of whether new neurons replace older ones (Becker 2005) or are added to the network (Aimone et al. 2009; Weisz and Argibay 2009)

remains unresolved. A recent quantitative analysis of post-natal neurogenesis in the macaque monkey dentate gyrus revealed that approximately 40% of granule neurons in a mature adult macaque are born post natally, with the 25% within the first 3 months after birth. In the same study, it was found that the size of the dentate gyrus keeps increasing even in mature animals (Jabes et al. 2010). Finally, two interesting aspects of the SGZ are that its activity is affected by gender, with females having higher basal levels of neurogenesis and showing fluctuations depending on the reproductive state (Galea and McEwen 1999; Westenbroek et al. 2004; Barker and Galea 2008) and by corticoid rhythms (Pinnock et al. 2007; Pinnock and Herbert 2008).

Homeostatic neurogenesis is significantly altered in the aged brain. In terms of structure, the ventral domains of the SEZ are gradually disappearing with ageing, leading to a smaller niche and lower numbers of generated cells (Luo et al. 2006; Blackmore et al. 2009). Similarly, the volume of the human hippocampus is reduced in the elderly (Small et al. 2002) and levels of neurogenesis in the SGZ are markedly decreased in the aged rodent and human brain (Heine et al. 2004; Lucassen et al. 2010). The decline in normal neurogenesis has been correlated with deficits in fine olfactory discrimination (Enwere et al. 2004) and cognitive deficits (Drapeau et al. 2003; Bizon et al. 2004). Very little is known about the mechanisms that underline this age-related decline in neurogenesis. It could be caused by a gradual exhaustion of the intrinsic self-renewal potential of NSCs (Amrein et al. 2011), to aberrant NSC maintenance signalling (affecting molecules such as leukaemia-inhibitory factor, wnt and notch signalling) (Lie et al. 2005; Bauer and Patterson 2006; Ferron et al. 2011), or to defects in cell cycle regulation (Kippin et al. 2005; Molofsky et al. 2006). Surprisingly, recent experimental work has revealed that NSCs within the aged SEZ retain their cardinal properties and behave similar to young NSCs in vitro (Ahlenius et al. 2009), that exercise can partially reverse the decrease in neurogenesis (van Praag et al. 2005; Blackmore et al. 2009) and that exposure to a young milieu (such as in parabiosis experiments) can rejuvenate adult progenitors either located within cytogenic niches (Villeda et al. 2011), or in the parenchyma (Ruckh et al. 2012).

2.2.4 Plasticity of Cytogenic Niches: Exploring the Limits

Under abnormal conditions, such as in response to degeneration or to external stimuli (as during episodes of stress or exercise), cell production in the SEZ and the SGZ can be significantly altered. This is manifested as changes in the numbers of cells generated and in the balance between neurogenesis and gliogenesis. The plasticity of these systems has also been tested—probably to the extreme—by exogenous manipulations, such as genetic interference or administration of growth factors and morphogens. Intracerebroventricular (i.cv.) infusion of epidermal growth factor increases astrogliogenesis not only in the SEZ and the adjacent striatum (Doetsch et al. 2002), but also within the olfactory bulb (Kuhn et al. 1997), while i.cv. infusion of fibroblast growth factor-2 enhances neurogenesis.

Both growth factors lead to significant increases in the size of the SEZ (Kuhn et al. 1997) an effect also elicited by i.cv. infusions of vessel endothelial growth factor (Jin et al. 2002). Pigment epithelium-derived growth factor and leukaemia inhibitory factor induce the self-renewal activity of NSCs (Bauer and Patterson 2006; Ramirez-Castillejo et al. 2006) while treatment with platelet-derived growth factor leads in increased oligodendrogenesis (Jackson et al. 2006). Interesting, albeit in many cases conflicting, results have also been generated by manipulating availability of brain-derived neurotrophic factor in the SEZ (reviewed in (Bath et al. 2012) and nitric oxide can be acting both to enhance or restrict neurogenesis depending on the context (reviewed in (Estrada and Murillo-Carretero 2005). The balance between neuro- and gliogenesis can also be altered by interfering with the molecular machinery of progenitors. Overexpression of transcription factors Pax6 and Olig2 enhances the generation of neurons and oligodendrocytes, respectively (Hack et al. 2005). Disruption of BMP signalling favours oligodendroglial over neuronal fate (Colak et al. 2008), while the transcription factor Sox9 was recently shown to promote self-renewal of NSCs and to regulate the balance in the generation of divergent cell fates (Scott et al. 2010). Moreover, disruption of microRNAs and of epigenetic modifications also result in changes in the level of neurogenesis (Liu et al. 2010; Szulwach et al. 2010).

In the SGZ neurogenic niche, even though the primary output is neurogenic, gliogenesis can be significantly enhanced by overexpressing the transcription factor Mash1 (Asc1) (Jessberger et al. 2008) and astrogliogenesis by knocking out reelin (Zhao et al. 2007). Infusion (i.cv.) of either fibroblast or epidermal growth factors does not induce proliferation in the hippocampus, but the latter results in a bias in differentiation in favour of gliogenesis (Kuhn et al. 1997). On the other hand, i.cv. administration of vessel endothelial growth factor leads to strong increase in proliferative activity within the SGZ (Jin et al. 2002).

2.2.5 Plasticity of Cytogenic Niches: Disease

Importantly, degenerative phenomena in the brain have been shown to induce the generation of neuronal subtypes not normally produced by SEZ progenitors, such as striatal spiny interneurons ectopically found in the striatum after stroke (Chmielnicki et al. 2004) and glutamatergic neurons in the injured cortex (Colak et al. 2008). In addition, astrogliogenesis has been reported to be enhanced after stroke (Li et al. 2010) and recent experimental work showed that migrating neuroblasts are diverted toward oligodendrogenic fate in areas of demyelination (Jablonska et al. 2010), thus widening the plasticity potential of SEZ progenitors to cells having exited the microenvironment of the niche that were previously thought to be almost irreversibly committed (Hack et al. 2005) (Figs. 4, 5). Another novel and surprising potential of NSCs residing in the SEZ that was recently demonstrated is the generation of ependymal cells either after their controlled chemotoxic ablation, or after their ageing-related destruction (Luo et al. 2008) (Fig. 5). This property of adult NSCs becomes even more interesting in light

of the evidence that after ischaemic injury, ependymal cells behave as progenitors; they migrate out of the niche and into the penumbra where they generate neurons and glia (Fushiki et al. 2003), a property that has also been attributed to ependymal cells lining the wall of the central canal in the spinal cord (Barnabe-Heider et al. 2010).

Experimental studies have shown that proliferation is significantly increased in the SEZ in response to traumatic brain injury (Gotts and Chesselet 2005; Ramaswamy et al. 2005) and focal ischaemic lesions that model stroke in humans (Li et al. 2001, 2010; Zhang et al. 2004) with many of these newly generated cells migrating towards the infracted areas (Arvidsson et al. 2002; Yamashita et al. 2006; Thored et al. 2007; Pluchino et al. 2008; Jin et al. 2010; Li et al. 2010). Stereological analysis of the rat SEZ after a focal cortical injury revealed that cell numbers in the niche increased from a total of 300,000 cells to a total of 450,000 cells in a 2 days time frame (Gotts and Chesselet 2005). Enhanced proliferation in the SEZ has also been observed in patients suffering from epileptic seizures (Grote and Hannan 2007) and multiple sclerosis (Picard-Riera et al. 2002; Nait-Oumesmar et al. 2007). Neurogenesis is increased in human cases and animal models of Huntington's disease (reviewed in (Curtis et al. 2011) while it is decreased in patients and animal models of Alzheimer's and Parkinson's disease (Hoglinger et al. 2004; Elder et al. 2006; Ziabreva et al. 2006; Curtis et al. 2007). The effects of exercise or environmental enrichment in SEZ activity are not still clear (Komitova et al. 2005a, b; Blackmore et al. 2009) but these external conditions seem to influence its response to ischaemia (Komitova et al. 2005a, b).

Status epilepticus strongly increases SGZ proliferation in short term, while leading to decreased activity long term (reviewed by (Parent et al. 2007) and ischaemia enhances proliferation but only of neurogenic progenitors (Tureyen et al. 2004). Importantly, the plasticity of the SGZ neurogenic system is demonstrated either as changes in proliferation, or in cell survival. Traumatic injury and enhanced physical activity significantly induce the proliferation of progenitors (van Praag et al. 2005; Urbach et al. 2008), but hippocampus-dependent learning promotes the survival of newly born cells (Leuner et al. 2004; Epp et al. 2007). A critical finding stemming from experimental work in animals was that antidepressants increase neurogenesis in the SGZ and that by either inhibiting this effect, or by depleting neurogenesis, the efficacy of the drugs is markedly decreased (Santarelli et al. 2003). Nevertheless, a recent study failed to reveal any effect of antidepressants in SGZ neurogenesis in elderly human patients (Lucassen et al. 2010). This observation highlights the difficulty in extrapolating experimental results to human conditions; difficulty that might be even bigger in the case of SGZ neurogenesis due to the effects of the endocrine system, as reflected in the role of the hypothalamic–pituitary–adrenal axis (Snyder et al. 2011) and of the gender (Barker and Galea 2008). Importantly, augmenting evidence indicates that the plasticity of hippocampal neurogenesis is tightly regulated by epigenetic modifications (Zhao et al. 2003; Parent et al. 2007; Ma et al. 2009).

3 Conclusions: Requirements—Limitations—Opportunities

Experimental animal work and descriptive studies in the human brain performed during the last two decades have clearly demonstrated that persistent neurogenesis in the adult brain is essential for normal functions, such as olfactory discrimination, learning and memory. What is still largely unknown is whether endogenous neurogenesis is or can be an important contributor to any therapeutic strategies regarding mental and cognitive disorders as well as to the response of the brain to degeneration. The fact is that adult neurogenesis in mammals is restrained within specific microenvironments and furnishes new neurons only in very specific target areas and neuronal networks. It also seems that numbers of surviving NSCs are significantly reduced with ageing, resulting in depleted cell reserves at the time when they are wanted the most. Moreover, as the brain became bigger and more complicated, during evolution, comparatively less NSCs survived in adulthood. On the other hand, recent analysis revealed that the capacity of adult and even aged NSCs to generate new cells can be markedly restored experimentally (in rejuvenation experiments, or with exercise and administration of growth factors). Even more, the observation that the majority of adult-born neuronal progenitors and neurons normally die before reaching their target and before maturation indicates that with the appropriate treatments, the overall output of adult cytogenic niches can be significantly improved. In the case of cognitive and mental disorders, small level manipulations might prove to be highly efficient since it would not be necessary to exogenously divert migratory routes and to re-specify commitment. In the case of tissue degeneration, it will be necessary to recruit small numbers of progenitors in novel (for them) directions (such as towards the substantia nigra in patients with Parkinson's), or to induce both higher levels of cell generation and a redirection of cells (such as after stroke, or in Huntington's disease) (Batista et al. 2006). Again, in order to have a measure of requirements and capacity, the loss of cortical neurons in a mouse model of Alzheimer's disease has been estimated at approximately 50,000 cells (Lemmens et al. 2011), while the enlargement of the SEZ cell population in response to stroke has been documented to reach even 150,000 cells (Gotts and Chesselet 2005). Nevertheless, in the end it might be proven that in certain degenerative conditions the most important contribution of adult brain cytogenic niches is not neuronal cell replacement, but rather the creation of a neuroprotective environment (Jin et al. 2010) possibly via interactions with the immune system (Cusimano et al. 2012).

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Neurogenesis and Hippocampal Plasticity in Adult Brain

Yan Gu, Stephen Janoschka and Shaoyu Ge

Abstract Plasticity in the adult brain enables lifelong learning. The fundamental mechanism of adult neural plasticity is activity-dependent reorganization of pre-existing structure, in contrast to the widespread cellular proliferation and migration that occurs during development. Whereas adult hippocampal dentate gyrus continuously generates cohorts of neurons, and newborn neurons integrate into the existing neural circuit under the regulation of existing global and local neural activity, demonstrating a unique cellular and synaptic flexibility in adult brain. Exhibiting an enhanced structural and synaptic plasticity during the maturation, adult-born hippocampal neurons may represent a unique population for hippocampal function. Current evidence indicates that lifelong addition of new hippocampal neurons may extend early developmental plasticity to adulthood, which continuously rejuvenates adult brain. We reviewed most recent advancements in exploring the circuit and behavioral role of adult-born hippocampal neurons.

Keywords Adult neurogenesis · Hippocampus · Dentate gyrus · Plasticity

Abbreviations

DG	Dentate gyrus
DGCs	Dentate granule cells
SGZ	Subgranular zone
LTP	Long-term potentiation
GABA	Gamma-amino butyric acid

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Curr Topics Behav Neurosci (2013) 15: 31–48
DOI: 10.1007/7854_2012_217

Published Online: 10 August 2012

NMDA N-Methyl-D-aspartate
AMPA Alpha-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid

Contents

1 Introduction: Hippocampus and Adult Neurogenesis

The hippocampus is one of the most extensively studied brain regions for synaptic plasticity and experience-modulated behavior. Numerous circuit tracing, targeted lesioning, and functional imaging studies have shown that the hippocampus is required for short-term declarative memory (Squire 1992), contextual associational memory (Rudy and Sutherland 1995), episodic memory (Vargha-Khadem et al. 1997), olfactory discrimination (Eichenbaum et al. 1989), and spatial navigation (O'Keefe and Dostrovsky 1971). A remarkable level of hippocampal plasticity has been proposed as a key factor in these functions. Central and long-standing hypotheses of the molecular and cellular mechanism of learning propose that memory formation relies on changes in synaptic strength and activity-dependent synaptic modification (Martin and Morris 2002; Neves et al. 2008).

Historically, the dentate gyrus (DG) has been characterized as the 'gate' of the *trisynaptic* hippocampal circuit (Hsu 2007), receiving perforant path projections from the neighboring neocortex and passing integrated information to the hippocampal CA3 subfield. Plasticity in this region has been found important for the function of hippocampus in many species including human. Based on anatomical, electrophysiological, and computer simulation data, it has been suggested that the DG plays an important role in learning and memory, including processing and representing spatial information on the basis of conjunctive encoding of multiple sensory inputs, pattern separation of spatial (especially metric) information, and temporal event integration in conjunction with the CA3 (Kesner 2007).

The subgranular zone (SGZ) of the DG is one of the only two widely acknowledged regions to date that retain neurogenesis under physiological conditions (Altman and Das 1965; Zhao et al. 2008). Continuous integration of new dentate granule cells (DGCs) provides another form of plasticity to the hippocampus in addition to the activity-dependent sub-cellular plasticity.

2 Structural and Functional Integration of Adult-Born DGCs

2.1 Structural Plasticity by Addition of New Neurons

In adult rodents, there are several thousand new neurons generated every day in the DG, modifying approximately 6 % of the total DGC population per month (Cameron and McKay 2001). Although most of these newborn DG cells (60–80 %) undergo programed cell death within 1 month following birth (Cameron and McKay 2001; Dayer et al. 2003), a remarkable number of new neurons survive and functionally integrate into the existing neural circuits (Ramirez-Amaya et al. 2006).

Hippocampal neurogenesis declines with age in mammals. It remains controversial whether ongoing adult neurogenesis provides a net increase in functional adult capacity or steady-state turnover of dying cells in the DG. Some studies have shown that the total number of DGCs does not increase with age in rat (Merrill et al. 2003; Rapp and Gallagher 1996; Rasmussen et al. 1996), whereas others indicate an increase in mice under different experimental conditions (Kempermann and Gage 1999; Kempermann et al. 1997). However, it is generally acknowledged that the elevated cell death observed in neurogenic active sites, including the DG, suggests that existing DGCs may be replaced or compete with newborn DGCs for survival (Biebl et al. 2000), and the rate of newborn cell addition is highly variable based on ontogenetic factors and behavioral activity.

A substantial number of newborn neurons integrate into the existing hippocampal neural circuits, suggesting that they are likely to play functional roles within local brain circuits and regional associated behavior. As new neurons integrate into the existing neural circuit for survival and function, they form new connections with afferent projections and efferent targets within the neural circuit. Therefore, continuous addition of new neurons in the DG introduces structural plasticity throughout the adulthood.

2.2 Functional Integration of Adult-Born Neurons

In 1984, Paton and Nottebohm for the first time characterized the synaptic activities of adult-born neurons in vivo in song birds, demonstrating that the newly generated neurons functionally integrate into the neural circuits and respond to

learned auditory stimuli (Paton and Nottebohm 1984). However, the sparse distribution and difficulty in direct visualization of these newborn neurons hindered the conclusive determination of many of their synaptic and functional properties. With the discovery that murine retrovirus selectively infects mitotic cells, it became possible to label and birth-date newborn neurons by virally introducing fluorescent protein genes into dividing neural stem cells in the DG. Pioneering work using this new technique was carried out by Gage's group on fluorescently labeled adult-born DGCs in live brain tissue in a series of elegant analyses on the morphological development and integration of the adult-born neurons (van Praag et al. 2002; Zhao et al. 2006).

Approximately, 80 % of dividing progenitors in the SGZ are directed to the neuronal fate and develop into dentate granule neurons. Cells targeted to the neuronal fate are highly plastic and active during the following several weeks as they migrate radially into the inner third of the granular layer. The extent of neuroblast tangential migration is currently unknown, although clonal analysis suggests such migration is minimal (Bonaguidi et al. 2011).

After complete development, newborn DGCs display typical morphology of dentate granule neurons, including apical dendritic trees projecting to the dentate gyrus molecular layer which form synaptic inputs from entorhinal cortical projections, and mature axons which contact neurons of the hilus and CA3 region.

2.2.1 Development of Adult-Born Neurons: Morphology and Membrane Properties

Recapitulating embryonic development, newborn DGCs in the adult brain follow a precise sequence of neuronal membrane development and synaptic connectivity before they become fully mature (Esposito et al. 2005; van Praag et al. 2002; Zhao et al. 2006).

During the first week, newborn DGCs have limited processes, spanning the granule cell layer toward molecular layer. All cellular properties resemble those of typical immature neurons of the developing brain, as they start to express neuronal sodium channels and fire immature action potentials.

At 2 weeks, new neurons have begun to migrate into the granule cell layer and to display typical granule cell morphology, with more numerous and elaborate dendrites traversing the molecular layer. No dendritic spines are observed at this stage. Membrane properties become more mature but still retain the characteristics of immature neurons.

At 4 weeks, newborn DGCs display morphology of mature granule neurons, including spiny dendrites that halt at the outer border of the molecular layer and axons that project to the CA3 region. Basic physiological properties mimic mature neurons at this stage, exhibiting mature action potentials and all known types of DGC synaptic connections, although synaptic plasticity continues to mature, as discussed below.

2.2.2 Formation of GABAergic Inputs

GABA has been shown to play crucial roles in regulating the development and synaptic integration of newborn neurons (Ge et al. 2007a).

Lacking synaptic inputs in the first week, newborn DGCs in the adult brain are tonically activated by ambient GABA. Functional GABAergic synapses that receive phasic GABAergic inputs from local interneurons start to form 1 week after birth (Ge et al. 2006), with the slow kinetics of GABA-activated currents indicating the initial formation of dendritic rather than perisomatic GABAergic synapses, which start to form 2 weeks later (Esposito et al. 2005; Ge et al. 2008). Physiologically, these GABAergic inputs to adult-born DGCs share the same characteristics of mature DGCs born in embryonic and early postnatal stages, and have similar functional properties (Laplagne et al. 2007).

GABA has an excitatory action owing to the high cytoplasmic chloride ion content of newborn DGCs in the first 2–3 weeks, and plays crucial role in regulating migration, development, and synaptic integration of newborn neurons (Ge et al. 2007a). Tonic GABA activation depolarizes newborn DGCs, and more importantly, it constitutes the majority of GABA-induced activation during the initial integration process when phasic GABA activation either does not exist or is weaker than tonic activation. Experimental conversion of GABA-induced depolarization (excitation) into hyperpolarization (inhibition) in newborn neurons leads to marked defects in synapse formation and dendritic development (Ge et al. 2006). Both the voltage-dependent and independent Ca^{2+}-permeable channels could be involved. Newborn DGCs in the adult brain express high levels of low-voltage-activated T-type Ca^{2+} channels which are activated below - 57 mV. Thus, tonic depolarization by GABA may lead to an activation of these Ca^{2+} channels and subsequent Ca^{2+} influx, leading to activity-dependent regulation of the development and integration of newborn neurons. However, detailed study will be needed to further determine the mechanism by which tonic GABA activation regulates these processes.

2.2.3 Formation of Glutamatergic Inputs

Following the formation of GABAergic synapses, glutamatergic inputs from the entorhinal cortex initiate synaptic connections on to the growing dendrites of adult-born DGCs. Although the first spines are formed approximately 2 weeks after birth and spine growth peaks at 3–4 weeks, further structural modifications continue for months (Zhao et al. 2006).

Recently, spine development on dendrites of newborn DGCs has been analyzed by 3D reconstruction of serial-section electron microscopy images (Toni et al. 2007). Filopodia—immature, motile protrusions that probe for potential synapse partners—extend very close to preexisting synaptic connections (within 200 nm) but not randomly, suggesting they are attracted to preexisting synapses, potentially by spillover glutamate from the active synapses (Kullmann and Asztely 1998;

Portera-Cailliau et al. 2003). Consistently, new dendritic spines are found in contact with multiple-synapse boutons, which contacts more than one postsynaptic spines. At 1 month, one-third of newborn spines contact single-synapse boutons, whereas all others contact multiple-synapse boutons. By the mature stage, two-thirds contact single-synapse boutons. These data suggests that newborn DGC spines initially form an preexisting synapses and later competitively replace preexisting postsynaptic spines as they stabilize (Toni et al. 2007), updating preexisting hippocampal neural circuit. By 4–8 weeks, adult-born DGCs display functional glutamatergic synaptic inputs similar to mature neurons (van Praag et al. 2002).

Glutamatergic inputs also regulate neurogenesis in the adult hippocampus, presumably by modulating neuronal integration and survival during development. Some studies have shown that AMPA receptor potentiation increases adult neurogenesis (Bai et al. 2003), while loss of NMDA receptor activity decreases newborn neuron survival (Tashiro et al. 2006). Seemingly, in contradiction, application of NMDA or AMPA receptor antagonists increase adult neurogenesis in the DG mainly by the regulation of cell proliferation (Bernabeu and Sharp 2000; Cameron et al. 1995; Gould et al. 1997). These results suggest that the regulation of adult neurogenesis by glutamatergic activity is complex, possibly through different downstream signaling pathways, or sensitive to environment or behavioral changes following treatment.

2.2.4 Formation of Synaptic Outputs

As newborn DGCs extend dendrites into the molecular layer, they extend axons rapidly toward the CA3 region. One week after birth, newborn axons pass through the hilus and reach the proximal CA3 region; by 2 weeks, they begin to form *en passant* expansions (Faulkner et al. 2008; Hastings and Gould 1999; Markakis and Gage 1999; Zhao et al. 2006). Axons continue to grow along the CA3 within 3–4 weeks while their expansions grow into larger, mossy fiber boutons (Faulkner et al. 2008). Newborn DGC axons do not extend beyond CA3, so they ultimately share the same trajectory as preexisting mature mossy fibers. According to the evidence provided by confocal and electronic microscopy studies, the earliest output synaptic contacts form on the dendritic shafts of target neurons by 2 weeks. Boutons grow in size and form spinous synaptic contacts, or share/compete spines with preexisting boutons (Faulkner et al. 2008; Toni et al. 2008). It takes 8–16 weeks for these new mossy fiber boutons to reach full maturity, with multiple invading dendritic spines and a stable number of synaptic contacts (Faulkner et al. 2008).

Recently, the excitatory optogene channelrhodopsin has been targeted into adult-born DGCs using retrovirus to study the functional synaptic output of these newborn neurons following light stimulation. The results indicate that mature adult-born DGCs establish functional synapses with hilar interneurons, mossy cells, and CA3 pyramidal cells and release glutamate as their main neurotransmitter, as do mature

DGCs (Toni et al. 2008). However, the complete process of axonal integration and maturation remains unclear. Furthermore, it will be interesting to determine whether the unusual diversity of interneuron targets reported as a distinguishing factor of adult-born olfactory neurons is also a feature of hippocampal neurogenesis (Bardy et al. 2010).

3 Circuit and Behavioral Roles of Adult-Born Neurons

3.1 Functional Relevance of Adult-Born Neurons

Since newborn neurons are continuously integrated into the existing hippocampal circuits throughout the adulthood, a key question is: do they contribute to known hippocampal function? Numerous studies using diverse approaches have shown the involvement of adult-born DGCs in hippocampal-dependent behaviors (Kee et al. 2007; Ramirez-Amaya et al. 2006; Tashiro et al. 2007).

Resulting from voluntary exercise (Farmer et al. 2004; van Praag et al. 1999), enriched environment (Nilsson et al. 1999), enhanced neurogenesis is usually associated with elevated synaptic plasticity in the DG and/or improved hippocampal-dependent learning and memory. A recent study also demonstrated that genetically increasing DG neurogenesis by specific inhibition of newborn cell death sufficiently improves hippocampal-dependent pattern separation (Sahay et al. 2011). Consistent with the hypothesis that neurogenesis has a positive role in learning and memory, decreased neurogenesis in either transgenic mouse lines, such as Methyl-CpG binding protein 1 knockout (MBD1-/-) mice, or ablation of neurogenesis by genetic methods or irradiation results in decreased synaptic plasticity in the DG and/or deficits in some forms of hippocampal-dependent learning and memory (Arruda-Carvalho et al. 2011; Drew et al. 2010; Imayoshi et al. 2008; Saxe et al. 2006; Snyder et al. 2001; Zhao et al. 2003).

Furthermore, Drapeau et al. observed a quantitative relationship between water maze performance, and the number of newborn neurons in the hippocampus of aged animals, in which animals that retained spatial memory exhibited a higher level of cell proliferation and a higher number of new neurons in comparison to those with spatial memory impairments (Drapeau et al. 2003).

Combining BrdU labeling of newborn neurons and immediate-early gene expression in active neurons, Frankland's group revealed the activity of adult-born DGCs in mice during behavioral tasks, indicating the incorporation of adult-born DGCs into special memory circuits (Kee et al. 2007). A recent study by his group selectively ablated a population of predominantly mature, adult-born neurons using a diphtheria toxin-based strategy without affecting ongoing neurogenesis. Removal of these integrated, adult-born neurons after learning degraded existing hippocampal-dependent contextual fear and water maze memories, suggesting that

adult-born neurons form a critical and enduring component of hippocampal memory traces (Arruda-Carvalho et al. 2011).

Together, these studies suggest that adult-born DGCs are involved in and required for normal hippocampal function. So the following question would be: do the adult-born neurons have unique properties and thus play unique roles in the hippocampal circuit?

3.2 Synaptic Plasticity of New Neurons

Synaptic plasticity such as long-term potentiation (LTP) has been thought to be the primary cellular basis of hippocampus-dependent learning and memory.

Wojtowicz's group found that immature DGCs in the inner granular layer, as determined by immature neuron marker TOAD-64 expression, are completely unaffected by GABAa inhibition and always display robust LTP with a lower induction threshold (Wang et al. 2000). This was confirmed by another group who found that DGCs expressing PSA-NCAM, another immature neuron marker, display immature properties, and have lower threshold for LTP induction and enhanced LTP (Schmidt-Hieber et al. 2004). Furthermore, by recording the evoked field potentials of populations of DGCs, Snyder et al. found a form of LTP could be induced in absence of GABAa receptor blocker picrotoxin. This LTP was selectively blocked by gamma irradiation 3 weeks before recording (Snyder et al. 2001), suggesting that this form of LTP is mediated by newly generated young neurons. Whereas the LTP mediated by mature neuron could only be observed in the presence of picrotoxin to block the local GABAergic inhibition.

Under physiological conditions, in intact neural circuit with GABAa inhibition in the adult brain, synaptic LTP plasticity in the DG appears to be largely dependent on young adult-born neurons. In contrast, mature DGCs display much less LTP in the same condition (Wang et al. 2000), or have higher threshold for LTP induction (Schmidt-Hieber et al. 2004).

3.3 Critical Window of Enhanced Synaptic Plasticity of Adult-Born Neurons

In classic experiments on the visual cortex, Hubel and Wiesel established the term "critical period" to describe a specific time window in which neuronal properties are particularly susceptible to modification by experience, concurrent with large-scale anatomical changes that become irreversible after closure of the time window (Hubel and Wiesel 1962; Wiesel and Hubel 1963). During this period, neurons display enhanced morphological and synaptic plasticity, and critical

period plasticity is now considered a central mechanism for establishing fine-tuned neuronal circuits in the developing brain (Hensch 2004).

Newborn neurons in the adult brain recapitulate embryonic neuronal developmental processes (Esposito et al. 2005), including proliferation, differentiation, functional integration, and maturation. During integration, these neurons start to receive experience-driven inputs from existing neural circuits. Do adult-born neurons also experience a critical period with enhanced synaptic plasticity, or are their synaptic properties maintained over a long period of time?

Using a retroviral approach for labeling and birth-dating adult-born DGCs, it became possible to study the plasticity of newborn neurons at specific ages. Ge et al. recorded from adult-born DGCs of different ages while stimulating the medial perforant pathway, followed by LTP induction using theta-burst stimulation. They found an enhanced LTP with decreased induction threshold in young adult-born DGCs at the age of 4–6 weeks that rapidly drops by 8 weeks of age (Ge et al. 2007b), indicating a critical period with enhanced synaptic plasticity. This is in consistent with other studies showing adult-born neurons display a high level of anatomical plasticity during this period which decreases thereafter, such as spine motility (Zhao et al. 2006), suggesting that the newborn DGCs undergo a short period of fine-tuning while integrating into existing circuits.

3.4 Molecular Mechanisms of Enhanced Synaptic Plasticity in Young Neurons

How are new neurons more plastic during this period? Lines of studies have shown that immature adult-born DGCs display distinct active and passive membrane properties such as high input resistance (van Praag et al. 2002). Moreover, in young neurons, high levels of T-type Ca^{2+} channels can generate isolated Ca^{2+} spikes and boost fast Na^{+} action potentials, contributing to the induction of synaptic plasticity (Schmidt-Hieber et al. 2004).

Another key mediator of plasticity is the N-methyl-D-aspartate (NMDA) type of glutamate receptors (NMDARs). During adult neurogenesis, NMDARs are expressed early, starting from immature neuronal stages (Carleton et al. 2003; Nacher et al. 2007).

It is known that during early postnatal neuronal development, switching of NMDARs subtypes from NR2B to NR2A changes the direction and degree of synaptic plasticity (Barria and Malinow 2005; Kim et al. 2005; Liu et al. 2004; Tang et al. 1999; Zhao et al. 2005). NMDARs containing NR2B subunit are expressed early during postnatal development and appear to be associated with enhanced synaptic plasticity during the critical period (Barria and Malinow 2005; Cull-Candy and Leszkiewicz 2004), while NMDARs containing NR2A, which are expressed and dominant later, mediate dramatically decreased LTP after the critical period (Barria and Malinow 2005).

Using field potential recordings, Snyder et al. revealed that LTP in the DG with intact GABAergic inhibition, which is potentially mediated by young adult-born neurons, could be specifically blocked by NR2B antagonist ifenprodil (Snyder et al. 2001). By specifically targeting adult-born DGCs, Ge et al. showed ifenprodil completely abolished LTP on DGCs of 4-weeks old, but not 8-weeks old or mature DGCs, providing a temporal correlation between synaptic expression of NR2B subtypes and critical period plasticity. They also found that the plasticity of newborn DGCs within the critical period rely significantly more on NR2B-containing NMDARs than on pan-NMDARs, suggesting that NR2B, which is the major NMDARs subtype expressed during the critical period, plays an instructive role in the enhanced synaptic plasticity of adult-born DGCs within this time window (Ge et al. 2007b). These studies suggest that adult-born neurons in the critical period undergo molecular mechanisms similar to neurons in the early postnatal critical period.

3.5 Contribution of Young Adult-Born DGCs to Hippocampal Functions

Since adult-born DGCs integrate into existing neural circuits of the hippocampus, and they express an enhanced synaptic and anatomic plasticity during the critical period in response to experience other than mature neurons that have passed the critical period (Ge et al. 2007b; Hensch 2004; Katz and Shatz 1996), do they make unique contributions to hippocampal function?

Emerging evidence suggests that adult-born DGCs might be preferentially recruited into hippocampal neural circuits that mediate novelty recognition, contextual fear conditioning, spatial information processing and memory formation (Denny et al. 2011; Kee et al. 2007; Ramirez-Amaya et al. 2006). This preferential recruitment appears at 4–6 weeks after birth, which is consistent with the critical period of the adult-born DGCs (Denny et al. 2012; Kee et al. 2007).

Owing to their high excitability (Mongiat et al. 2009; van Praag et al. 2002) and critical period plasticity for the fine-tuning of synaptic incorporation in the neural circuitry in response to experience, adult-born DGCs of the critical period are more readily recruited into the hippocampal circuit for the encoding of novel information. As modeled by Aimone et al., the special properties of young adult-born neurons are required for the formation of temporal clusters which associate individual elements of long-term episodic memories (Aimone et al. 2006).

Overall, the available evidence strongly indicates that young adult-born neurons play an important role in participating in certain types of hippocampus-related behaviors, particularly learning and memory. Based on the expression of doublecortin (DCX) or CRMP4, proteins specific to immature neurons, the population of young adult-born neurons could correspond up to 10 % of all granule cells of the DG, and are likely to have a broad impact on the entire hippocampus.

Therefore, by continuously generating cohorts of new neurons, adult hippocampus is able to retain an enhanced form of plasticity in a population of DGCs for the function of hippocampus throughout the adulthood.

4 Regulation by Local and Global Neural Activity

Activity-dependent anatomical reorganization is widely regarded as a fundamental mechanism of developmental and adult neural plasticity. Adult neurogenesis is similarly dynamic and highly dependent on the activity of the neural circuits. As DG receives various innervations from multiple brain regions, adult-born neuron development at distinct stages is regulated by numerous factors related to global and local neuronal activities.

As previously mentioned, neurogenesis in the adult hippocampus is significantly enhanced by an enriched environment (Brown et al. 2003; Kempermann et al. 2002; Kempermann et al. 1997), and physical activity (running) (Brown et al. 2003; Farmer et al. 2004; Rhodes et al. 2003; van Praag et al. 1999). Gould and colleagues reported that spatial learning activity increased the number of newborn neurons in the adult hippocampus (Gould et al. 1999). Electric stimulation in the entorhinal cortex or LTP induction in the DG enhanced both proliferation and survival of adult-born neurons (Bruel-Jungerman et al. 2006; Stone et al. 2011). Recent evidence showed different activity level in the septal and temporal pole of the hippocampus results in the difference of the integration and maturation of newborn neurons. Newborn neurons in the septal pole of DG display higher rate of maturation as indicated by intermediate early and neuronal marker gene expression, morphology, and electrophysiological properties, possibly due to higher septal pole basal network activity (Piatti et al. 2011). NMDA receptor-dependent hippocampal learning activity has been shown to promote the survival of specific populations of adult-born neurons (Dupret et al. 2007; Gould et al. 1999). In fact, loss of NMDA receptor activities decreases the survival of adult-born neurons (Tashiro et al. 2006). In general, neural circuit activity regulates adult neurogenesis in the brain, and NMDA receptor activation appears to be critical for not only proliferation of newborn neurons, but also successful integration and survival of new neurons by strengthening the correct functional glutamatergic synaptic connections through activity-dependent synaptic modification.

On the other hand, ambient GABA levels, regulated by interneuron activities, may also serve as a general indicator of dynamic neuronal network activity in a sparsely activated DG. Before receiving any synaptic innervations, newborn neurons may sense local neuronal network activities through ambient GABA. The maturation of GABAergic synapses is associated with the critical developmental period of adult-born DGCs. Thus, the tempo of the synaptic integration and the critical period of the newborn neurons are regulated by local GABAergic inputs (Ge et al. 2008).

Also importantly, as adult-born DGCs begin to express muscarinic and nicotinic acetylcholine receptors and receive direct cholinergic innervations early in their development, their successful integration and function are regulated by the activity of these inputs (Campbell et al. 2010; Harrist et al. 2004; Mohapel et al. 2005). Lesion of the medial septum, which sends cholinergic projections to the DG, decreases the survival of newborn neurons (Van der Borght et al. 2005). Similarly, selective neurotoxic lesion of forebrain cholinergic input to the DG also reduces adult neurogenesis (Cooper-Kuhn et al. 2004; Mohapel et al. 2005), possibly as a result of increased apoptosis of newborn DGCs (Cooper-Kuhn et al. 2004). Consistent with these results, systemic administration of the cholinergic agonist physostigmine or acetylcholinesterase inhibitor donepezil increases neurogenesis by enhancing survival of newborn DGCs (Kaneko et al. 2006; Kotani et al. 2006). Proliferation of adult neural hippocampal progenitors is also reduced in mice lacking the beta2-subunit of the nicotinic acetylcholine receptor (Harrist et al. 2004). Likewise, knockout of the alpha7 subunit of the nicotinic acetylcholine receptor not only decreases the survival of the newborn neurons, but also affects their maturation. The neurons of both alpha7-knockout mice and those infected with retrovirus which knocks down alpha7 mRNA display a prolonged period of GABAergic depolarization characteristic of an immature state (Campbell et al. 2010).

Furthermore, increased serotonergic signaling increases adult neurogenesis (Banasr et al. 2004), while blockage decreases it (Brezun and Daszuta 1999). Adult neurogenesis is also regulated by dopaminergic, norepinephrine, and NO systems (Hoglinger et al. 2004; Jhaveri et al. 2010; Zhang et al. 2001). In addition, modulation of excitatory neurotransmission by hormonal release in certain conditions, such as stress, is known to shift hippocampal neurogenesis, dendrite remodeling, and synaptic capacity (McEwen 2007).

In summary, the generation, survival, maturation, and integration of adult-born DGCs are precisely regulated by global and local neural circuitry activities, depending on the environmental conditions and the developmental stage and receptor expression of the newborn neurons.

5 Conclusion and Open Questions: The Unique Plasticity of Hippocampal Circuits

During the initial development, adult-born neurons display distinct properties, such as high input resistance, high structural plasticity, and enhanced susceptibility for the induction of LTP before they are fully matured.

Due to the enhanced excitability and plasticity, young adult-born DGCs provide unique capability to local circuits and associated behaviors. It has been proposed that adult-born DGCs might be highly engaged as the DG integrates memory of events that occur within a narrow window of time (Aimone et al. 2006; Deng et al. 2010).

Thus adult neurogenesis may provide not only a source of replacement neurons for maintenance of hippocampal structure, synaptic connections, and steady DGC number, but also an ongoing developmental process that continuously rejuvenates the mature nervous system by offering expanded capacity of plasticity in response to experience throughout life so that the adult brain maintains the ability to adapt to new experiences.

New evidence is emerging that adult-born neurons are also important for other hippocampal-related functions. Recently, an additional temporal function was reported showing that newborn neurons modulate the transference of short-term fear memory to hippocampus-independent memory centers, a process potentiated by voluntary exercise (Kitamura et al. 2009). Interestingly, Feng et al. found that forebrain-specific presenilin-1 knockout mice, which have a deficiency in enrichment-induced neurogenesis in the DG, did not show appreciable learning deficits, but a marked increase of memory retention after post-learning enrichment, suggesting that adult dentate neurogenesis also plays a role in the periodic clearance of outdated hippocampal memory traces after cortical memory consolidation, thereby ensuring that there are young newborn DGCs in the hippocampus continuously available for processing new memories (Feng et al. 2001). New approaches are required for further clarification, particularly to disambiguate the functional contributions of the newborn neurons in the adult brain.

Evidence also shows the difference of neurogenesis along the septo-temporal axis of the hippocampus. Development of newborn neurons is accelerated at the septal pole, possibly due to higher septal pole basal network activity (Piatti et al. 2011). The septal pole of the hippocampus is known to be associated with spatial learning and memory while the temporal pole with affective behavior, but the significance of these findings is not yet fully understood, especially since this same study reported increased physiological activity due to running preferentially activates temporal pole development.

However, the full functions of adult-born DGCs are not yet fully understood, nor is it entirely clear why a cell-based reorganization strategy is retained in the hippocampal archicortex but not the neocortex. While we currently have a far stronger understanding of the diverse factors which influence this unique form of brain plasticity, further studies and new approaches, particularly, in coordination with the results of olfactory neurogenesis studies, will be required to address these important questions.

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Adult-Born Neurons in the Olfactory Bulb: Integration and Functional Consequences

Gilles Gheusi, Gabriel Lepousez and Pierre-Marie Lledo

Abstract The generation of new neurons is sustained throughout life in the olfactory system. In recent years, tremendous progress has been made toward understanding the proliferation, differentiation, migration, and integration of newborn neurons in the olfactory bulb. Here, we discuss recent findings that shed light on different aspects of the integration of adult-born neurons into olfactory circuitry and its significance for behavior.

Keywords Adult neurogenesis · Brain plasticity · Olfaction · Learning · Memory · Behavior · Neural stem cells

Contents

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Curr Topics Behav Neurosci (2013) 15: 49–72
DOI: 10.1007/7854_2012_228

Published Online: 14 September 2012

1 Introduction

One underestimated property of the adult nervous system is its lifelong ability to undergo structural and functional remodeling. Among the various mechanisms underlying adult neuroplasticity is the renewal of neurons in some restricted areas. Because neurogenesis was traditionally viewed as a matter of brain development (embryonic and perinatal stages), the pioneering work by Altman and colleagues (Altman 1962; Altman and Das 1965) that provided the first evidence for newly generated neurons in the adult rat had no major impact at that time (Gross 2000, 2009). We know today, far from doubt, that two discrete regions of the adult brain are the sites of production of new neurons: the sub-ventricular zone (SVZ) lining the walls of the lateral ventricles and the sub-granular zone (SGZ) in the dentate gyrus (DG) of the hippocampus (Kriegstein and Alvarez-Buylla 2009; Lledo et al. 2006; Ming and Song 2005; Zhao et al. 2008). In this chapter, we will focus on adult neurogenesis that impacts the olfactory system, and therefore odor-guided behaviors.

The olfactory bulb (OB) continuously receives adult-born neurons through life from the SVZ. Neuroblasts travel tangentially toward the OB along a path of migration known as the rostral migratory stream (RMS) (Lois and Alvarez-Buylla 1994), where they travel in chains through tubular structures formed by astrocytes. Once the neural precursors reach the OB circuit, they detach from their migratory chain to migrate radially toward the periphery of the OB where they mature into olfactory interneurons of two types: granule cells (GCs) and periglomerular cells (PGCs). Ultimately, each of these two classes of interneurons form functional synapses with the OB's projection neurons, the mitral and tufted cells. Neuroblasts migrate radially in close association with blood vessels (Bovetti et al. 2007; Snapyan et al. 2009; Saghatelyan 2009). Adult neurogenesis is a multistep process that involves the production of cell progenitors, their migration, their differentiation and maturation into fully integrated neurons. It should be kept in mind that most of our knowledge about the neurogenic events in the adult brain comes from studies that have been carried out on laboratory rodents. However, variations among mammalian species still must be considered to get more insight into the importance of adult mammalian neurogenesis (Bonfanti and Peretto 2011).

Compelling evidence supports the notion that observations made previously in rodents stands true also for human beings. For instance, using different tools, several studies have reported the presence of newly generated neurons in adult human OB (e.g., Liu and Martin 2003; Bédard and Parent 2004). Newly generated neurons in humans may modulate olfactory discrimination in a similar way as reported in rodents. This may come as a surprise to many people, though not to those who make their living by their noses, including enologists, perfumers, and food scientists. Anyone who has taken part in a wine tasting, or observed professional testing of food flavors or perfumes, knows that the human sense of smell has extraordinary capacities for learning how to discriminate odorants, especially after a never-ending training. Nevertheless, a recent finding by Sanai et al. (2011)

has casted some doubts on this conjecture. By showing the lack of robust tangential migration from the human SVZ to the OB, the authors were prompted to call into question the status of adult neurogenesis for the human OB. So, for the human case we are left with the widely accepted notion that the SVZ indeed exists in the adult brains (see for instance Bernier et al. 2000; Freundlieb et al. 2006; Tepavcevic et al. 2011) and that human OB, despite its small size, contains newly generated granular and periglomerular interneurons that express a wide variety of chemical phenotypes (see for instance Bédard and Parent 2004; Maresh et al. 2008). Also, supporting the latter is the finding that the adult human OB core is a reservoir of neural precursor cells and pluripotent neural stem cells (Liu and Martin 2003). What remains unclear is how newly generated neurons reach the adult human OB (for a recent review, see Sierra et al. 2011) and how long adult-born neurons survive in human OB (Bergmann et al. 2012).

1.1 Synaptic Organization of the OB Network

Olfactory sensory neurons (OSN) located in the olfactory epithelium project to the periphery of the OB. Each OSN sends an axon to the OB where the axon projects to interneurons and projection cells (Firestein 2001). In contrast to the neocortex, the OB circuitry is characterized by a large proportion of inhibitory inputs to projection neurons. The majority of adult-born neurons are GABAergic (GCs and PGCs), with a small percentage of dopaminergic neurons (PGCs). Both form dendro-dendritic synapses with mitral and tufted cells (Shepherd et al. 2004). GCs represent approximately 95 % of OB interneurons. They are axonless and project a long dendrite with numerous branches through the GC layer (GCL) that forms dense dendro-dentritic synapses with mitral and tufted projection neurons. In response to firing of mitral and tufted cells, at the dendro-dendritic synapse glutamate is released onto GC spines which in turn triggers GABA release onto activated mitral cell and tufted cell dendrites. This mechanism that supports the recurrent inhibition also mediates lateral inhibition between neighboring mitral cells and allows mitral cell synchronization during odor presentation (Rall and Shepherd 1968; Friedman and Strowbridge 2003; Schoppa 2006; Geffen et al. 2009). Finally, bulbar interneurons also receive inhibitory inputs from other types of local OB interneurons (Eyre et al. 2008) and excitatory synapses from axons collaterals of mitral cells and tufted cells and from terminals of centrifugal projections (Balu et al. 2007) (Fig. 1).

1.2 The Neurogenic Processes that Govern the Production of Newly: Generated Neurons in the Subventricular Zone

In the embryo, bulbar interneurons are generated in the ganglionic eminence (Wichterle et al. 2001) and then migrate ventrally to reach the developing OB. Quantitatively, this early prenatal neuronal production is rather minor when

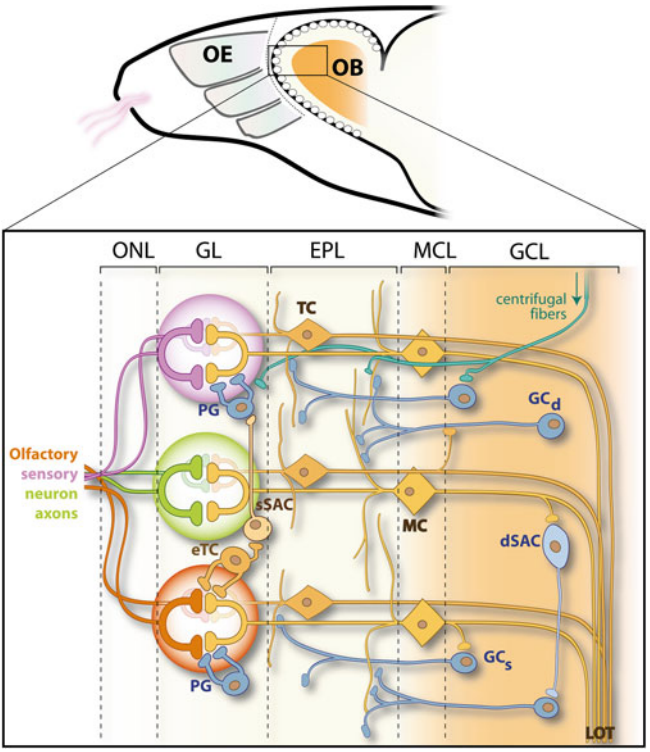

Fig. 1 Anatomical organization of the olfactory bulb. *Top panel*. Schematic diagram of the mouse head. The olfactory epithelium (OE) located in the nasal cavity is composed of sensory neurons which project to the olfactory bulb (OB). *Bottom panel*. Olfactory sensory neurons (OSNs) in the olfactory epithelium (OE) that express the same odorant receptors project their axons to the main olfactory bulb into one of the glomeruli that form the Glomerular Layer (GL). In the GL, sensory neuron terminals synapse onto the apical dendrites of output neurons, namely the mitral cells (MC) and the tufted cells (TC). In addition, periglomerular cells (PGC), superficial short-axon cells (sSAC), and external tufted cells (eTC.) act on glomerular synaptic transmission exerting diverse functional effects. In the external plexiform layer (EPL), the lateral dendrites of mitral and tufted cells interact with the dendrites of granule cells (GC). Granule cells can also be subdivided into distinct subpopulations: superficial granule cells (GC_S) that target the superficial lamina of the external plexiform layer and synapse with tufted cells. Deep granule cells (GC_D) targeting the deep lamina of the external plexiform layer are connected to mitral cells. The soma of mitral cells are aligned and delineate the Mitral Cell Layer (MCL), and the soma of tufted cells are scattered in the EPL. Granule cell somas and also some deep short-axon cells (dSAC) compose the granule cell layer (GCL). Centrifugal fibers from others' brain regions innervate specific layers of the olfactory bulb, with respect to their brain origin. Lastly, output neuron axons fasciculate to form the lateral olfactory tract (LOT). All the cell types colored in orange are glutamatergic, GABAergic cells are in blue. ONL, olfactory nerve layer

compared to the one achieved postnatally. After the animal's birth, bulbar interneurons are derived from a region dorsal to the ganglionic eminence called the SVZ because it is placed near the lateral ventricles of the forebrain. There,

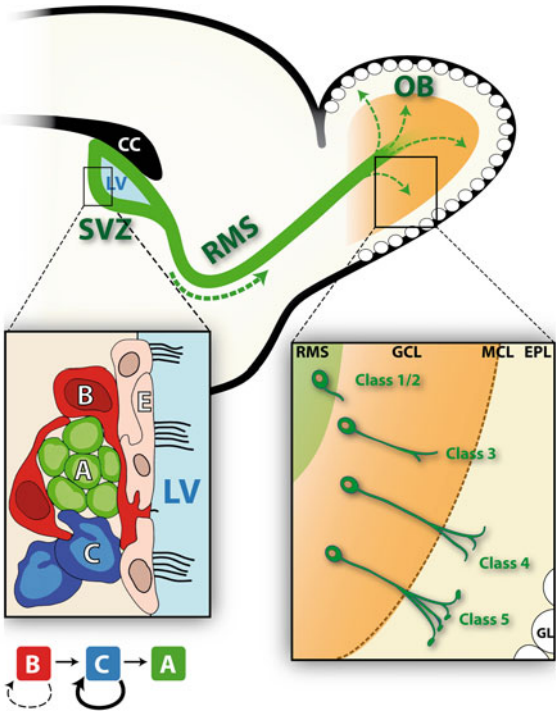

Fig. 2 The subventricular zone—olfactory bulb neurogenic system of the adult mouse. *Top panel* schematic diagram of a sagittal section of the mouse forebrain. The subventricular zone (SVZ) lies in the walls of the lateral ventricle (LV), below the corpus calosum (CC). After their generation in the SVZ, neuroblasts migrate tangentially along the RMS to their final destination in the olfactory bulb (OB). *Bottom left panel* schematic diagram of the SVZ neurogenic niche. Separated from the lateral ventricle by a monolayer of ependymal cells (E), slow-dividing astrocytic stem cells (*red*, type B cells) divide to generate transit-amplifying cells (*blue*, type C cells), which in turn give rise to neuroblasts (*green*, type A cells) that start to migrate in chain to the rostral migratory stream (RMS). *Bottom right panel* morphological maturation sequence of adult-born neurons. From the migrating neuroblast in the rostral migratory stream (RMS; 5 days after birth), young cells sequentially differentiate into interneurons (mainly granule cells, here represented in green). Cell maturation is characterized by a progressive growth of the apical dendrite toward the external plexiform layer (EPL). GCL, granule cell layer. MCL, mitral cell layer

thousands of newly formed neurons migrate daily in the RMS to reach the OB (Temple and Alvarez-Buylla 1999) (Fig. 2). Within the SVZ, the neural precursor cells are considered to be genuine stem cells since not only do they proliferate but also they give rise to several different cell types (Temple and Alvarez-Buylla 1999). Unlike other immature neurons produced during embryogenesis, SVZ precursor cells migrate tangentially in chains without the need of radial glial cells and continue dividing during their migration to populate the OB where they differentiate into local interneurons. Detailed analysis of both in vitro and in vivo results support the notion that the adult SVZ hosts neural stem cells are capable of

producing the three major cell types of the central nervous system: neurons, astrocytes, and oligodendrocytes, with the vast majority becoming neurons (about 95 %). When neuroblasts reach the OB, they migrate radially and of those that survive, approximately 95 and 3 % differentiate into GABAergic and dopaminergic interneurons, respectively. It is still debated why the excitatory–inhibitory balance at the first central relay has to be tuned so precisely. Since the largest neuronal population in the OB consists of GABAergic GCs that outnumber the output neurons (mitral and tufted cells) by a factor of more than 10 (Shepherd et al. 2004), and one GC contacts hundreds of mitral or tufted cells, and each mitral or tufted cell, in turn, contacts a large number of pyramidal cells of the pyriform cortex, the very upstream location of the recruitment of adult-born neurons in the OB appears ideal for amplifying the neurogenic effect throughout the entire olfactory pathway. Below we discuss some of the recent findings that support functional implications of adult-born neurons in sensory information processing.

1.3 Maturation and Integration of Adult-Born Neurons in the Olfactory Bulb

Adult-born interneurons are faced with the challenge of integrating into fully functional circuits. The key molecular and cellular events that govern the synapse formation, development and integration of newly formed neurons into the OB circuitry have begun to be elucidated only recently. Taking advantage of the development of genetic advances such as the selective labeling of newborn neurons and pre- and post-synaptic terminals, some studies have described the sequence of the maturation and the synaptogenesis of adult-born neurons in the OB. The use of retroviruses (Petreanu and Alvarez-Buylla 2002) when combined with some mouse strains that allow a specific expression of GFP in nestin- or doublecortin-expressing cells, for instance, results in specific labeling of immature neurons in the OB as well as in the DG (Mignone et al. 2004; Wang et al. 2007). This approach allows a detailed morphological description of all the developmental stages of adult-born neurons as they integrate into the OB circuit. Petreanu and Alvarez-Buylla (2002) were the first to describe five successive stages of GC maturation, from migrating neuroblasts having a single leading process to mature GCs with a complex dendritic arbor. Class-1 cells represent cells migrating in the RMS. When they reach the OB, newborn neurons (class-2) begin their radial migration and extend their apical dendrite through the GCL toward the mitral cell layer. Around 10 days after their birth, newborn neurons first receive GABAergic synaptic input (class-3) before they receive excitatory (glutamatergic) input a few days later (class-4). Finally, adult-born neurons reach their final stage of maturation (class-5) after 4 weeks of development when they exhibit distal branches with full spine density. Class-3 GCs receive proximal GABAergic and glutamatergic inputs (see Kelsch et al. 2008; Panzanelli et al. 2009; Katagiri et al. 2011). In

turn, newborn class-4 GCs develop output synapses (i.e. the dendro-denritic synpases) that they establish with the lateral dendrites of projection neurons (mitral and tufted cells) (Kelsch et al. 2008). A recent study suggests that although the synaptic outputs of newborn GCs emerge during the first 2 weeks of development, the maturation of functional GABA release is slow since it continuously increases several weeks after the cell's birth (Bardy et al. 2010). As previously reported in the developing brain, between 28 and 56 days after their birth, newborn GCs exhibit a transient overproduction of spines followed by a drastic elimination (Whitman and Greer 2007). Finally, it is worth noting that all these steps are rather slow when compared with the time frame of embryogenesis. A recent study demonstrating that the highly dynamic rearrangement of spine density in adult-born GCs occurs several months after their birth (Livneh and Mizrahi 2011) adds further support to this notion.

Most of the knowledge we are reporting here comes from studies that have been carried out on rodents. However, some reports have revealed striking differences in the features of adult neurogenesis in other mammalian species (for a recent review see Bonfanti and Peretto, 2011). These differences concern the organization of the neurogenic niche and the rostral migratory stream (Quinones-Hinojosa et al. 2006; Ponti et al. 2006; Rodriguez-Perez et al. 2003; Sanai et al. 2004; Smart et al. 2002), as well as the timing of neuroblast migration and differentiation (Kornack and Rakic 2001; Brus et al. 2012). Thus, direct comparison of the different features of adult neurogenesis between species that differ in behavioral specialization and ecological adaptation is critical to our functional understanding of the continued production of adult-born neurons (Amrein et al. 2011; Baker et al. 2011).

1.4 Adult-Born Interneurons Form Synapses in an Activity-Dependent Manner

The neuronal connectivity of adult-born neurons that integrate into OB circuitry is highly sensitive to changes of the level of excitability of the cells. This has been demonstrated in studies aimed at challenging the neural activity in a cell-autonomous manner (Kelsch et al. 2009; Lin et al. 2010) or the level of sensory inputs by odor deprivation and/or exposure to an enriched olfactory environment (Mizrahi 2007; Kelsch et al. 2009; Livneh et al. 2009; Livneh and Mizrahi 2011). Sensory deprivation decreases synaptic wiring of adult-born neurons and this sensory input-dependent change is restricted to a time window when adult-born neurons first develop their synapses (Sagathelyan et al. 2005; Kelsch et al. 2009). Surprisingly, the different regions of the GC dendrite are differently affected by a reduction of sensory input. While the distal and basal parts of the GC dendrite show a reduction of excitatory inputs, the density of the glutamatergic synapses on the proximal region of the apical dendrite increases. This dual regulation could represent a compensatory mechanism to preserve a minimal level of excitation on

adult-born GCs and therefore for survival. In addition, cell-intrinsic excitability interacts with sensory-driven inputs. Using genetic tools (expression of a bacterial voltage-gated sodium channel—NaChBac), Lois and colleagues demonstrated that the increase of intrinsic excitability of adult-born GCs counterbalances the sensory deprivation-induced changes and promotes synaptic integration of newborn cells (Kelsch et al. 2009; Lin et al. 2010).

Undoubtedly, neural activity plays a critical role in the formation and maintenance of synapses in newborn GCs. Both intrinsic neural activity and afferent inputs shape the function of input and output signals of adult-born cells during a limited period of time. The idea that adult neurogenesis produces neurons that are transiently plastic has recently received support from slice physiology. By investigating the characteristics of proximal glutamatergic inputs onto GCs, it has been shown that a form of synaptic plasticity (i.e. long-term potentiation) occurs during a restricted period of time: between 2 and 6 weeks after the cell birth (Nissant et al. 2009). However, the view that activity-dependent modulation of GCs synaptogenesis strictly occurs during a restricted and brief period has been recently challenged. Using in vivo time-lapse two-photon microscopy, Livneh and Mizrahi (2011) showed that both GCs and PGCs are capable of undergoing experience-dependent plasticity well after their maturation and integration into the OB. This long-lasting form of plasticity may represent a strategy to stabilize fine-tuned neuronal circuits in response to changes in the sensory environment. Remarkably, it is also expressed by the adult-born neurons of the DG (Lemaire et al. 2012). Defining the specific functions brought by adult-born neurons will necessitate comparing their potential for synaptic reorganization over extended periods of time with that of neurons generated during neonatal development. This has been recently examined by Kelsch and colleagues (2012). Compared with neonatal GCs that show a large degree of variability in the density of their synaptic inputs, adult-born interneurons display stable synaptic connectivity over time. One potential functional consequence of these findings is that neonatal and adult-born GCs may make differential contributions to the circuit in response to sensory experience.

1.5 Activity-Dependent Survival of Adult-Born Neurons

One half of the new interneurons that are added in the adult OB integrate and survive (Petreanu and Alvarez-Buylla 2002; Winner et al. 2002) and it is now clearly established that sensory experience influences their survival (Petreanu and Alvarez-Buylla 2002; Rochefort et al. 2002). Elimination occurs during a critical period of time, ranging from 14 to 28 days after cell birth (Yamaguchi and Mori 2005), and depends on centrifugal inputs engaged during sleep (Yokoyama et al. 2011 see also below). Interestingly, this time window in GC development coincides with the period of maturation for glutamatergic input synapses (Carleton et al. 2003; Kelsch et al. 2008; Whitman and Greer 2007).

The mechanisms that participate in the life–death decision remain still largely unknown. There is mounting evidence that apoptosis takes place after the newborn neurons receive excitatory inputs originating from axon collaterals of projection neurons, as well as from centrifugal projections of the olfactory cortex and before the adult-generated neurons form their GABAergic output synapses. Kelsch and colleagues (2008) have suggested that the excitatory inputs onto adult-generated neurons might link cell survival with the level of global excitation before the newborn neurons establish their dendro-dendritic output synapses with mitral and tufted cells. These sequential steps suggest an interesting framework to understand how synaptic maturation and sensory experience converge to regulate the survival of adult-born neurons. Notably, odor enrichment and odor learning promote the rate of survival and the integration of newborn neurons in the OB (Rochefort et al. 2002; Miwa and Storm 2005; Bovetti et al. 2009; Moreno et al. 2009; Veyrac et al. 2009). Conversely, a blockade in odor signal transduction in sensory neurons, naris occlusion, or a diazepam-enhanced inhibition in the olfactory circuitry decreases the survival of newly generated neurons (Corotto et al. 1994; Petreanu and Alvarez-Buylla 2002; Yamaguchi and Mori 2005). Odor enrichment involves noradrenergic-dependent mechanisms that promote the survival of olfactory newborn neurons (Veyrac et al. 2009). These results are supported by the rich noradrenergic innervation that the OB receives from the *locus coeruleus* (Shipley et al. 1985; Nicholas et al. 1993) and the positive effect of noradrenaline on newborn neuron survival (Bauer et al. 2003). One form of implicit memory, perceptual learning, refers to the improvement of one's ability to discriminate differences in the attributes of simple stimuli (Gilbert et al. 2001). Olfactory perceptual learning has been shown to increase the number of newborn GCs (Moreno et al. 2009) and a recent report argues that noradrenaline is required for olfactory perceptual learning (Moreno et al. 2012). Olfactory associative learning also promotes the survival of adult-born neurons in the OB (Alonso et al. 2006; Mouret et al. 2008; Kermen et al. 2010; Sultan et al. 2010, Sultan et al. 2011a, b). Importantly, learning-induced increase in neuronal survival has been observed for 18–30-day-old adult-born neurons, with the older cells dying (Mouret et al. 2008; Mandairon et al. 2006). This time window corresponds to the period during which newborn neurons begin to receive glutamatergic inputs (Kelsch et al. 2008) that undergo LTP (Nissant et al. 2009). Again, olfactory learning has been shown to result in significant increase in the levels of noradrenaline from the centrifugal projections of the *locus coeruleus* in the OB (Rosser and Keverne 1985; Lévy et al. 1990; Brennan et al. 1998; Sullivan et al. 2000). In a recent paper, Sultan et al. (Sultan et al. 2011a, b) confirmed the participation of newborn neurons in the network responsible for processing learned odorants. Interestingly, they also reported that newborn neurons are removed from the network when the memory trace is no longer active, demonstrated by breaking the odor-reward association (extinction). These data indicate that survival of adult-born neurons is also modulated by odor memory erasure.

The signaling pathways that lead to the elimination of adult-born neurons or that promote their survival have not been elucidated. This is an important topic to

be investigated because of its relevance to cell-based functional regeneration in non-neurogenic regions after injury and degenerative neurological diseases. However, the 'decision' to die, or not, depends on the expression of apoptotic and anti-apoptotic proteins, respectively Bax and Bcl-2 (Kim et al. 2007; Miwa and Storm 2005). One question arises whether the elimination/survival processes of newly-formed neurons in the OB has a functional meaning. Using a caspase inhibitor to locally prevent elimination of newborn neurons in the OB, Mouret et al. (2009) rescued a significant number of new neurons from apoptosis and reported a deficit in odor exploration and odor discrimination in mice. This finding provides evidence that the continuous elimination of new neurons is not just a simple homeostatic mechanism but rather contributes to improving cognitive functions.

Despite accumulating evidence for the contribution of neurotrophic factors and neurotransmitters to the regulation of neuroblast proliferation and integration (Bovetti et al. 2011), our knowledge about the events that control life-and-death decisions of adult-born neurons is still rudimentary. Overall, three interrelated pathways mediate the survival of adult-born neurons: sensory experience-triggered dendro-dendritic synaptic changes, top–down glutamatergic inputs originating from cortical regions such as the olfactory cortex, and centrifugal modulation by neuroamines and neuropeptides locally released in the OB (Fig. 3). A recent and interesting finding partly illustrates this view. Yokoyama et al. (2011) reported that the number of apoptotic adult-born GCs dramatically increased within a few hours after eating in food-restricted mice compared to ad libitum fed mice. In addition, the number of apoptotic newborn GCs was positively correlated with the total length of sleep that occured during the postprandial period. Outside the feeding period, sleep *per se* did not have a potent effect on GC apoptosis. However, the sequence of feeding followed by sleep constituted an event that enhanced the elimination of newly formed granule cells. Intriguingly, blocking the access of sensory inputs to the OB potentiated GC apoptosis during the postprandial period. The authors suggested that during the awake state, when mice forage and eat food, olfactory experience activates a subset of adult-born GCs that will be rescued from death by a 'reorganizing signal' that enters the OB during the postprandial period. Conversely, without sensory experience, newly-generated GCs will be eliminated. The identity of the 'reorganizing signal' was not identified by the authors in this study, however, they suggested possible candidates, such as blood-circulating hormones, cholinergic and catecholaminergic neuromodulatory signals, and excitatory synaptic inputs from the olfactory cortex. Although these findings raise a number of interesting questions, the model suggested by the authors illustrates the possibility that a combination of events and mechanisms may determine the survival and integration of new GCs.

Because the size of the OB mouse does not change throughout life (Biebl et al. 2000; Petreanu and Alvarez-Buylla 2002), the continuous arrival of newborn interneurons in the olfactory circuitry suggests that adult neurogenesis is a matter of replacement rather than addition. However, perinatal-born neurons survive for much longer periods than adult-born GCs (Lemasson et al. 2005). Furthermore,

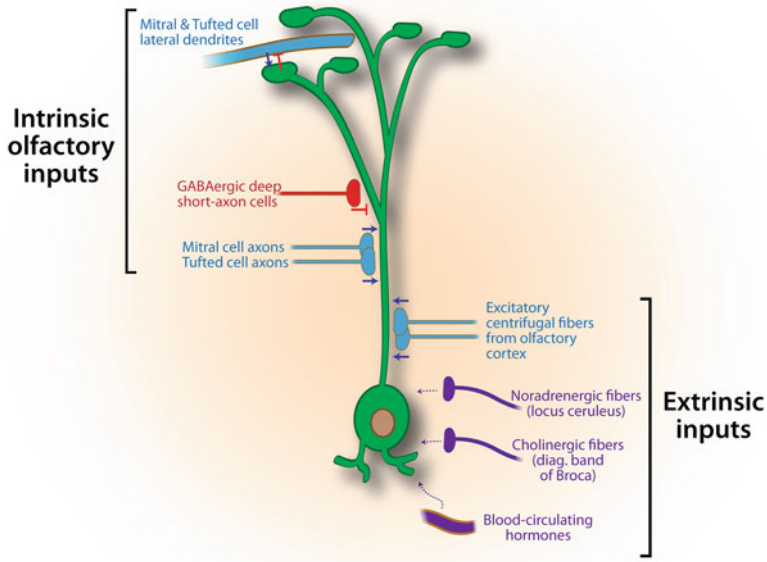

Fig. 3 Intrinsic versus extrinsic inputs regulate granule cell activity and survival. Adult-born granule cells are targeted and influenced (1) by different intrinsic neurons of the olfactory bulb network ("intrinsic olfactory inputs"), (2) by centrifugal fibers originating from different distant brain regions and by blood-circulating hormones ("extrinsic inputs"). During cell maturation, adult-born granule cells first receive somatic inputs from centrifugal fibers before establishing dendro-dendritic synapse with mitral/tufted cells. *Blue*, glutamatergic excitatory synapse. *Red*, GABAergic inhibitory synapse. *Violet*, neuromodulatory and hormonal inputs

they tend to reside in the superficial part of the GCL while adult-born GCs reside in the deepest layers (Lemasson et al. 2005; Mouret et al. 2008; Imayoshi et al. 2008). Imayoshi et al. (2008) reported that almost the whole population of deep adult-born neurons is continuously replaced by new neurons over a period of 12 months.

1.6 The Potential Functions of Olfactory Bulb Adult Neurogenesis

After confirming initial evidence for the existence of stem cells that generate new neurons in the adult brain, the key question to be addressed has been: What is the functional significance of the integration of adult-born neurons in the OB? As we have seen above, olfactory experience (odor enrichment, odor learning) can regulate the maturation and survival of adult-born neurons. Because newborn neurons

exhibit specific properties different from pre-existing interneurons (enhanced synaptic plasticity during a critical time window), it is generally thought that they should make a unique contribution to odor processing. One simple way to assess the functional contribution of adult neurogenesis is to ablate adult-born neurons. Different approaches have been developed to abolish adult neurogenesis. They include the use of anti-mitotic drug, such as cytosine arabinoside (AraC) (Doetsch et al. 1999), focal irradiation (McGinn et al. 2008), or conditional transgenic ablation of neural precursor cells (Singer et al. 2009). A complementary strategy to examine the functional contribution of adult-generated neurons includes recruiting new neurons during specific tasks. This can be done by using an immunohistochemical approach to examine whether adult-born neurons that have incorporated the mitotic analog bromodeoxyuridine (BrdU) also express immediate early genes, thereby indicating that they are functionally integrated into the circuitry during the specific behavior.

Perhaps one of the major functions of adult neurogenesis, acting in concert with centrifugal fibers, is to improve plasticity of neuronal networks. Several pieces of evidence support this hypothesis. First, Alonso and colleagues (2006) showed that olfactory experience is not sufficient to promote the survival of adult-born neurons. Only when animals could associate a given olfactory cue with a reward (or lack of) was survival increased (Alonso et al. 2006). Second, an electrophysiological study shows that a subset of glutamatergic inputs impinging onto adult-born GCs, presumably originating from the olfactory cortex, exhibit LTP when the synaptic transmission is activated by theta burst stimuli (Nissant et al. 2009). This study also found that theta burst stimulation induced LTP at newborn but not in mature GCs in the mouse OB.

Studies on the functional significance of adult OB neurogenesis have yielded many conflicting results. The contribution of adult OB neurogenesis to odor detection thresholds has been examined in two recent studies. Using a sniffing attraction task that consisted of recording the time spent by a subject freely investigating an odorant, Breton-Provencher et al. (2009) reported that mice treated with Ara-C showed higher detection thresholds and thus a reduced sensitivity of their odorant perception. In contrast, Lazarini et al. (2009) did not find any impairment of odorant perception in SVZ-irradiated mice trained to detect odors during a nose poke-based go/no-go odor-discrimination task. Some studies have reported that reduced neurogenesis impairs odor discrimination (Gheusi et al. 2000; Enewere et al. 2004; Bath et al. 2008). In contrast, other studies showed ablating neurogenesis in the OB did not interfere with odor discrimination (Imayoshi et al. 2008; Breton-Provencher et al. 2009; Lazarini et al. 2009; Sultan et al. 2010). Those studies and others have also attempted to examine the causal relationship between adult neurogenesis and olfactory learning. Alas, they have yielded mixed results. Following an almost complete ablation of adult neurogenesis, both in the SVZ and in the hippocampus, Imaysohi et al. (2009) reported no impairment of olfactory short-term memory in mice. These results contrast with those describing a reduction of short-term memory performance in Ara-C-treated mice submitted to a non-associative olfactory task (Breton-Provencher et al. 2009).

In the same vein, the short-term strength of odor-cue fear-conditioned olfactory memory has been shown to depend on adult olfactory neurogenesis (Valley et al. 2009). There is also no clear consensus from studies that have examined the involvement of adult olfactory neurogenesis in olfactory long-term memory. Indeed, one study showed that adult bulbar neurogenesis is required for the long-term retention of reward-associated odors (Sultan et al. 2010). Another study reported that long-term olfactory memory performance was slightly reduced in SVZ-irradiated mice compared to controls (Lazarini et al. 2009). Finally, two studies showed that animals with genetic and pharmacological ablations of adult bulbar neurogenesis performed normally in an odor-associated long-term memory task (Imayoshi et al. 2008; Breton-Provencher et al. 2009). The functional contribution of adult olfactory neurogenesis in a peculiar form of learning, i.e. perceptual learning, has also been studied. Perceptual learning is experience-dependent enhancement of one's ability to respond to the environment. Thus, in the olfactory system, perceptual learning refers to the improvement of the capacity to discriminate between odors following repeated exposure to these odorants. One of the basic questions about perceptual learning concerns the underlying neural mechanisms. This question has been addressed by Moreno et al. (2009) in the context of previous studies that have established that odor enrichment both increases one's ability to discriminate between odorants (Wilson and Stevenson 2003) and improves the survival of adult-born interneurons in the OB (Rochefort et al. 2002; Bovetti et al. 2009). The authors confirmed that odor enrichment increased the survival of adult-born olfactory interneurons. This supply of newly-generated neurons enhances the inhibition in the OB network and facilitates the ability of animals to discriminate between similar odors to which they have been previously exposed during the odor enrichment period. More importantly, they demonstrated that the pharmacological blockade of OB neurogenesis during odor enrichment prevents the improvement of olfactory discrimination in response to odor enrichment. These findings have led some authors to propose that adult neurogenesis in the OB, as well in the DG, constitutes a mechanism for improving pattern separation, i.e. the process that transforms similar objects into more discriminable stimuli (Sahay et al. 2011).

It should be noted that all of these models have some drawbacks which limit the interpretation of functional analyses due to non-specific effects of the manipulation. Ara-C, for instance, has been shown to diffuse away from its site of injection and to also reduce the survival of hippocampal neurons (Breton-Provencher et al. 2009; Moreno et al. 2009). Irradiation also affects oligodendrocyte cell precursors (Panagiotakos et al. 2007) and neurovascular cells (Coderre et al. 2006) and induces strong microglial activation for several weeks (Monje et al. 2002). Finally, even if transgenic mouse models allow the ablation of neural precursor cells with cellular specificity and temporal precision, they suppress neurogenesis in both the SVZ and the DG that severely limits the interpretation of behavioral analyses (Garcia et al. 2004; Imayoshi et al. 2008; Singer et al. 2009).

Overall, as described above, we are faced with a growing collection of studies that have investigated the functional contribution of adult olfactory neurogenesis

to odor-guided behaviors and have provided conflicting results. The methods of ablation, the timing and length of newborn neuron depletion, the possible compensatory mechanisms, and the different specifics of experimental design represent numerous factors that may account for the discrepancies between studies that have attempted to demonstrate a causal relationship between learning and adult olfactory neurogenesis.

In addition to the cognitive functions discussed above, some studies have examined the role of adult olfactory neurogenesis in some ethologically-relevant contexts (parental behavior, mate choice, mate odor recognition). Despite the major role played by olfaction in many species of mammals in their socio-ecological behavior, the number of ethologically minded studies that have focused on the functional contribution of adult olfactory neurogenesis to the natural behaviors of animals are still scarce (Gheusi et al. 2009). There are several reports on the ability of adult olfactory neurogenesis to respond to social experience. For instance, an increase in cell production in the SVZ, along the RMS, and in the subependymal zone of the OB was reported in female prairie voles exposed to a male conspecific (Smith et al. 2001). Huang and Bittman (2002) also showed that newborn cells in the main and accessory olfactory bulbs OB of male hamsters were activated following the exposure to an estrus female, female hamster vaginal secretions, or an aggressive male. In female mice, exposure for 7 days to male odors also promoted an increase in newborn cells in the SVZ, in the main OB (MOB), and in the accessory OB (AOB) (Mak et al. 2007; Larsen et al. 2008; Oboti et al. 2009; 2011; Nunez-Parra et al. 2011). Collectively, these findings suggest that adult-born olfactory neurons provide a substrate for the expression of a variety of social behaviors in rodents. This issue has been addressed by different authors who have also blocked adult neurogenesis, using the same means as those described above, and examined whether the expression of odor-guided social behaviors were altered. Mak et al. (2007) reported that the urine of a dominant but not a subordinate male caused an increase in the rate of proliferation of adult-born cells in the SVZ and in the DG that are stimulated by prolactin and the luteinizing hormone, respectively. In addition, dominant-male odor stimulation induced a preference for a dominant male over a subordinate male in a choice test. Interestingly, the blockade of adult neurogenesis prevented dominant preference in Ara-C treated females that were pre-exposed to dominant-male pheromones. These results provided convincing evidence for the role of adult neurogenesis in the context of mate selection in female mice. However, in this study the Ara-C treatment suppressed both bulbar and hippocampal neurogenesis, therefore the relative contribution of OB and hippocampus neurogenesis toward female mouse mate selection is still unknown.

Adult-born neurons may participate in behaviors surrounding pregnancy. Soon after mating, exposure of female mice to male urine odors different from the stud male promotes the interruption of embryo implantation, an effect called pregnancy-block or the Bruce effect (Bruce 1959). The failure of the mating male's chemical to block the pregnancy of a female provides evidence that the female can distinguish the odor of her mate from those of alien males to which she was not

exposed at mating. These findings show that female mice learn and recognize the odor of their mate after mating. The Bruce effect is mediated by male urine odors that are conveyed through the vomeronasal organ which projects to the AOB (Lloyd-Thomas and Keverne 1982). As stated above, the exposure of female mice to male urine odors increase the number of new GCs that survive in the AOB (Oboti et al. 2011). Interestingly, the low-molecular-mass urinary molecules that convey the effect of the pregnancy-blocking chemosignal (Peele et al. 2003) have also been identified as those which play a role in increasing AOB neuronal survival (Oboti et al. 2011). In order to know whether the male odor-stimulated neurogenesis in the adult AOB of female mice was the substrate for the pregnancy-blocking effect, the authors blocked the renewal of newly-generated interneurons in female mice by infusing Ara-C during 4 weeks before mating and re-exposed these females to their mating partner. In these conditions, stud male odors induced pregnancy-block in female mice indicating that newborn neurons in the AOB are required for the memory formation of the stud male urine odors during mating. It has been now established that, among the numerous morphological and functional changes that occur during motherhood, the production of adult-born neurons participates in maternal behavior (Leuner et al. 2010; Lévy et al. 2011; Larsen and Grattan 2012). Two waves of increased proliferation within the SVZ, one occurring during the early period of pregnancy and the other during the first week of lactation, has been described in mother mice (Shingo et al. 2003). These productions are stimulated by the hormone prolactin. An increase of production of neuroblasts from the SVZ occurs only during the last week of pregnancy in rats (Futura and Bridges 2005). Interestingly, virgin female mice exposed to a male or its urine show enhanced maternal behavior, and this effect has been shown to be mediated through activation of prolactin receptors (Larsen et al. 2008). Conversely, exposure to female pheromones suppresses the wave of increased cell proliferation normally observed in pregnant female mice (Larsen et al. 2010). It remains unclear whether the enhancement of cell proliferation in the SVZ observed in mice during the early-postpartum is a general phenomenon shared across mammalian species. However, it seems unlikely. For instance, Brus et al. (2010) recently examined the regulation of neural cell proliferation in the SVZ of adult nulliparous ewes and reported that parturition and interactions with their offspring downregulate cell proliferation in the SVZ and the DG. The increased cell proliferation observed during the early period of pregnancy and lactation in female mice has led to the hypothesis that it might support some functional aspects of maternal behavior, e.g. the onset and maintenance of maternal behavior, pup recognition, maternal aggression, etc. This has been investigated in two recent studies that have examined the consequences of the ablation of olfactory neurogenesis on maternal behavior in mice (Fereistein et al. 2010; Larsen et al. 2010). A selective reduction of OB neurogenesis by focal irradiation of the SVZ affected neither the onset and maintenance of maternal behaviors, nor the ability of mothers to discriminate their own pups from alien pups. Similarly, no impairment in the expression of maternal behaviors has been reported following a reduction of olfactory neurogenesis using an anti-mitotic drug or after a suppression of

prolactin secretion in female mice. Taken together, these results provide little evidence for a functional contribution of adult-born olfactory neurons to the establishment and expression of maternal behavior and pup recognition in female mice. Interestingly, the study by Wei et al. (2011) also reported no effect of the ablation of adult neurogenesis in adulthood on the display of maternal behavior. However, blocking neurogenesis during the juvenile period did induce an impairment of the ability of virgin female mice to retrieve pups. Two studies reported a deficit of maternal behavior in mice treated with an antimitotic drug and in transgenic mice with genetically ablated newly born neurons (Larsen et al. 2010; Sakamoto et al. 2011). Unfortunately, because SVZ and SGZ were blocked in both models, it remains unknown how much olfactory and hippocampal neurogenesis contribute respectively to the expression of maternal behaviors. A study has also examined the involvement of olfactory neurogenesis in paternal behavior in mice (Mak and Weiss 2010). Male mice housed for the entire duration of pregnancy and birth showed enhanced cell proliferation in the SVZ and the DG compared to fathers separated from their pups and their partner immediately after birth. Physical interaction for 2 days with own pups, but not alien pups, was required to stimulate neurogenesis in the paternal SVZ and DG. These effects were mediated by prolactin since blocking prolactin activity inhibited the upregulation of cell proliferation in the neurogenic niches of the paternal brain. From a functional point of view, the authors reported that the adult-born neurons generated when fathers interacted with their pups postnatally also preferentially responded to the odors of their adult offspring compared to non-offspring. Finally, the authors demonstrated that preventing the rise of neurogenesis via a blockade of prolactin activity in the paternal brain disrupted the ability of fathers to recognize their offspring. Altogether, these results provide a strong evidence of the functional contribution of adult neurogenesis in the context of fatherhood. Significant questions still remain to be addressed regarding the specific role of olfactory and hippocampal neurogenesis in offspring recognition in male mice and to the mechanisms by which biological pups, but not unrelated ones, are the source of the upregulation of neurogenesis in the father's brain.

All the research we discussed above highlights the importance of giving a critical evaluation to the strengths and weaknesses of the methodology used to investigate the functional significance of adult-born neurons. None of these methods can fully and exclusively block the birth of adult-born olfactory neurons in a spatially and temporally manner. Recently, new optogenetic tools have been added to the set of techniques available to researchers. Optogenetic approaches constitute a promising strategy to investigate the role of adult-born neurons. Optogenetics provides the opportunity to activate or silence a specific cell type in a temporally and spatially precise manner in freely-moving animals and could consequently elucidate the role played by that cell type in different behaviors. It is based on genetically encoded molecules that couple light and neural function. Using this approach to investigate the functions of adult-born neurons avoids many limitations and complications (nonselective ablation, compensatory mechanisms)

associated with the different forms of physical elimination of newborn cells discussed above. In a recent study, we selectively expressed channelrhodopsin-2 (ChR2) in adult-born GCs and showed that light activation of these newborn neurons facilitated difficult odor discrimination learning and improved odor memory (Alonso et al. 2012). Interestingly, accelerated acquisition of a difficult odor discrimination task required that stimulation be delivered at a high (40 kHz) rather than low (10 kHz) frequency and simultaneously with odor delivery. In addition, a positive correlation was found between the number of adult-born ChR2-positive GCs and the animals' performance. Finally, this experiment demonstrated that adult-born GCs have a unique function that could not be performed by their mature counterparts at the behavioral level. Indeed, activating adult-born neurons, but not early postnatal-born neurons, only results in a beneficial effect on odor discrimination learning and olfactory memory. Taken together, these results provide evidence of a direct and immediate causal contribution of adult-born olfactory neurons on the functioning of the olfactory circuits and its behavioral outcomes.

2 Conclusions

The acceptance that adult neurogenesis occurs in the adult brain constitutes a major step in the field of neuroscience. This form of metaplasticity is sensitive to a wide range of experiences that occur throughout the lifespan of individuals. Since the initial discovery in the 1960s, the field of adult neurogenesis has delineated significant questions, developed methodological advances, and established new perspectives on the plasticity of the adult brain. Consequently, a rapidly expanding literature has generated a large amount of reports over the past decade about the molecular biology of neural stem cells, differentiation, migration, and functional integration of adult-born neurons. Major steps have been reached in the description of the sequence of the synaptic integration of adult-born neurons. Future studies about the understanding of the role played by cortical inputs on the maturation and integration of newborn cells will be critical to increase our knowledge about the physiological regulation of adult neurogenesis. Following a period of correlative evidence between adult-born neurons production and behavior, especially learning and memory, major breakthroughs in methodological approaches have been made to unravel the functional contribution of olfactory neurogenesis. However, despite significant effort, the precise role played by the production of newly-generated neurons is still unclear due to the shortcomings inherent to each strategy employed as well as the specifics of experimental design and paradigms. Furthermore, we know very little about the contribution of specific ages of newborn neurons. For these reasons, new techniques and new approaches that selectively act on adult-born neurons, and even different sub-types of newborn neurons, in a precise temporal and spatial manner will be critical to fully elucidate the role of olfactory neurogenesis.

Acknowledgments We are particularly indebted to Matt Valley for his critical reading and comments about the manuscript. This work was supported by the foundation pour la Recherche Médicale, the Agence Nationale de la Recherche (ANR-BLAN-SVE4-LS-110624 and ANR-09-NEUR-004 in the frame of 'ERA-NET NEURON' of FP7 program by the European Commission, the Letten Foundation and the life insurance company 'Aprionis'.

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Network, Cellular, and Molecular Mechanisms Underlying Long-Term Memory Formation

Mariana Carasatorre and Víctor Ramírez-Amaya

Abstract The neural network stores information through activity-dependent synaptic plasticity that occurs in populations of neurons. Persistent forms of synaptic plasticity may account for long-term memory storage, and the most salient forms are the changes in the structure of synapses. The theory proposes that encoding should use a sparse code and evidence suggests that this can be achieved through offline reactivation or by sparse initial recruitment of the network units. This idea implies that in some cases the neurons that underwent structural synaptic plasticity might be a subpopulation of those originally recruited; However, it is not yet clear whether all the neurons recruited during acquisition are the ones that underwent persistent forms of synaptic plasticity and responsible for memory retrieval. To determine which neural units underlie long-term memory storage, we need to characterize which are the persistent forms of synaptic plasticity occurring in these neural ensembles and the best hints so far are the molecular signals underlying structural modifications of the synapses. Structural synaptic plasticity can be achieved by the activity of various signal transduction pathways, including the NMDA-CaMKII and ACh-MAPK. These pathways converge with the Rho family of GTPases and the consequent ERK 1/2 activation, which regulates multiple cellular functions such as protein translation, protein trafficking, and gene transcription. The most detailed explanation may come from models that allow us to determine the contribution of each piece of this fascinating puzzle that is the neuron and the neural network.

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Curr Topics Behav Neurosci (2013) 15: 73–115
DOI: 10.1007/7854_2012_229

Published Online: 13 September 2012

Abbreviations

Ach	Acetycholine
AP5	(2R)-amino-5-phosphonovaleric acid
BDNF	Brain-derived neurotrophic factor
BRs	β-adrenergic receptors
CA1	Cornu ammonis 1
CA3	Cornu ammonis 3
CaMKII	Ca^{2+}/calmodulin-dependent kinase II
cAMP	Cyclic AMP
CatFISH	Compartmental analysis of temporal activity using fluorescent insitu hybridization
CBP	CREB binding protein
CNS	Central nervous system
CREB	cAMP response element-binding
D1	Dopamine receptor 1
D2	Dopamine receptor 2
DAG	Diacylglycerol
DG	Dentate Gyrus
Elk-1	E twenty-six (ETS)-like transcription factor 1
EPSP	Excitatory postsynaptic potential
ERK	Mitogen-activated protein kinase
GABA	Gamma-aminobutyric acid
GFP	Green fluorescent protein
GluR1	AMPA receptor subunit 1
H3	Histone 3
IEG	Immediate early gene
IP3	Inosito trisphosphate
LPT	Long-term potentiation
LTD	Long-term depression
M1	Muscarinic acetylcholine receptors 1
M2R	Muscarinic acetylcholine receptors 2
M3	Muscarinic acetylcholine receptors 3
M4R	Muscarinic acetylcholine receptors 4
MAP2	Microtubule-associated protein 2
MAPK	Mitogen-activated protein kinases
MEK	Mitogen-activated protein kinase kinase
MK801	Dizocilpine
MSK1	Mitogen- and stress-activated kinase 1
NCAM	Neural cell adhesion molecule
NE	Endo-neuraminidase
NGF	Nerve growth factor
NMDA	N-Methyl-D-aspartic acid
NR 1	NMDA receptor subunit 1
NR 2	NMDA receptor subunit 2
NT	Neurotrophins

ODNs	Oligodeoxy nucleotides
PKA	Protein kinase A
PKC	Protein kinase C
PLC	Phospholipase C
PSD-95	Postsinaptic density protein 95
RSK2	Ribosomal protein S6 kinase 2
Ser/Thr kinase	Serine–threonine kinase
Tg	Transgenic
TrkB	Tyrosine kinase or BDNF/NT-3 growth factors receptor
Wave3	Actin nucleating factor

Contents

1 Introduction

Our learning or the information we acquire comes in diverse ways, either as explicit information that describes objects, places, or events or as implicit information that modulates behavioral responses and outcomes. Each different form of learning is processed by a particular neural network distributed throughout different brain regions. However, the mechanisms underlying these different information-processing tasks use basically the same network and cellular principles (Gilbert et al. 2001), and they may even use similar signal transduction pathways. Today, neuroscientists are generating a clearer picture of these underlying principles that help explain the cognitive processes of information acquisition and long-term memory formation. In this review we attempt to provide a general picture of network, cellular, and molecular mechanisms underlying long-term memory formation.

2 How does the Neural Network Work?

Features of the environment are detected by our sensory receptors that transduce these fragments of physical stimuli into patterns of neural activity. This process can be seen as an analog–digital information conversion; it represents the first limit

of our cognition and is determined by the characteristics of the sensory receptors of each species. Thus, the information we acquire depends on the features of the stimulus that can be extracted with our sensory detectors. But later, once the information is transduced into neural activity, the features of the stimuli are processed through distributed neural networks or circuits and later integrated in a holistic representation. In this way, the reality we perceive results from a reconstruction that our brain produces and depends on the features that were extracted from the environment and how they were integrated, which in turn depend on the way we have trained our brain to perform these information-processing tasks. These tasks use both serial and parallel neural pathways to achieve proper integration; importantly, however, all the processing occurs in *populations* of neurons, generating what is currently known as a population code (Sakurai 1998).

The theoretical proposal suggesting that objects or ideas are represented by the patterns of activity generated in groups of neurons was formally presented by Hebb (1943) in his book "The Organization of Behavior". He suggested that groups of neurons, which he called "neural ensembles" that possess recurrent connections, such as the ones described by Lorente de Nó (1933, 1938a, b), have the ability to maintain the newly acquired patterns of neural activity through reverberation. Hebb then suggested that this reverberatory activity leads to changes in the efficacy of synapses, a phenomenon that we currently call "synaptic plasticity". The idea is that synaptic plasticity stabilizes the ability of the cell assembly to reproduce the patterns of neural activity long after they were acquired through experience. The reproduction or reactivation of the activity patterns can occur either offline, which is in the absence of the stimuli, or online, which is in response to the original, a partial, or a distorted stimulus. Hebb′s ideas inspired the generation of computational neural network models that developed into the theory of the "attractor memory neural networks" (Lansner 2009).

The fundamental idea behind the computational models of "attractor memory neural networks" is basically the same; changes in the strengths of synaptic connections are required to stabilize the ability of the cell assembly to reproduce the neural activity patterns acquired through experience. These models integrate experimental observations obtained from different levels of analysis, as suggested by Marr (1982), and can display information-processing capabilities during simulation, such as associative memory and holistic perception (Mari 2004; Lansner 2009; Akrami et al. 2012). The models that integrate many biologically characterized variables, when designed with Hebbian synaptic changes can become self-organizing attractor neural networks models, which may be considered as the building blocks of neural network dynamics. These models provide the basic operations of the neural network, such as analog–digital conversion, categorization, association, and autoassociation (Akrami et al. 2012). These operations then turn into more sophisticated forms of cognitive tasks such as perception, learning, and memory or even language. The main operations displayed by these models also agree with Marr's original suggestion that association and statistical pattern comparisons were the principal information-processing mechanisms of the neural network (Marr 1971). Several self-organizing attractor neural networks models

create a stable dynamic attractor with which the network can reproduce the neural activity patterns acquired earlier during experience. Neural ensembles formed in this way will subsequently serve as the elements responsible for reproducing the patterns of neural activity acquired during experience, and these activity patterns are actually the neural representation of the stimulus (Barlow 1972). If at any time the external stimuli or cues occur in a distorted or fragmented way but place the network in the basin of the attractor, the network dynamics will drive the neurons to reproduce (to some extent) the activity patterns that were exhibited during acquisition of the original experience. This is called pattern completion, and it is the mechanism through which the network reactivates the originally acquired activity pattern in response to partial or distorted information. Pattern completion explains holistic perception or what is also known as "the gestalt phenomenon", while lateral inhibition will mediate perceptual rivalry in case of ambiguity between the stimuli and explains pattern separation.

Although Hebb's ideas were generally correct, some important details were missing, and artificial recurrent neural network models initially had trouble achieving reverberatory activity (MacGregor and McMullen 1978; Lansner and Fransén 1992; Wilson and Bower 1992). These original models used a dense and symmetric connectivity that requires high-frequency neural activity to function properly (Hopfield 1982), in clear contrast to what has been described physiologically in the mammalian brain (Lansner 2009). But later, the models of recurrent neural networks integrated important biological features from cortical pyramidal cells, such as modular organization and functioning (Lansner 2009). Examples of these modules in the biological neural network, are the cortical columns (Mountcastle et al. 1967; Mountcastle 1997; Hubel and Wiesel 1959, 1962) or the clustered striosome–matrisome in the striatum (Amemori et al. 2011), and can also organize without clustering cells (Redish et al. 2001) but instead apparently in a modular dendritic branch composition (Ascoli et al. 2009). The models that integrate a modular architecture present a sparse and asymmetric connectivity at the neuron-to-neuron level, and symmetrical function at the mini column level (Lansner 2009). This symmetrical function of neuronal modules is now widely used to develop complex neural network models that can achieve sophisticated cognitive tasks. Other biological features that were integrated are synaptic features provided by glutamatergic transmission, particularly *N*-methyl-D-asparate (NMDA) receptor function (Lansner and Fransén 1992; Traub et al. 1992), and the modulatory role of GABAergic interneurons (Mariño et al. 2005; Rudolph et al. 2007). These new models have reliably reproduced reverberatory activity in the neural network simulations and are able to properly achieve the cognitive endeavors.

Experimental findings of delayed persistent activity after stimulation (Romo and Salinas 2003; Lemus et al. 2007; Barak et al. 2010), neural activity associated with memory retrieval (Jacobs et al. 2012), and the existence of modules (or columns in the cortex) with feature selectivity (Mountcastle et al. 1967; Mountcastle 1997; Hubel and Wiesel 1959, 1962) suggest that memory as a whole consists of the integration of distributed features or pieces of the represented stimuli. One very

important advantage of this type of modular architecture is that newly acquired patterns of activity can build new representations on the foundation of network attractors acquired previously from other representations (Mari 2004). This fascinating idea was originally suggested by Jean Piaget (1954) and is currently being supported by neural network models. In the most basic computational models, memory retrieval occurs when the partial or distorted stimulus evolves into a pattern strongly correlated with one of the representations stored in the synaptic weights (Hopfield 1982). This is a universal mechanism for memory retrieval and the correlated activity of the reciprocally connected modules is potentially very relevant (Mari 2004).

The main factors that determine the effectiveness of attractor dynamics are: connectivity, representation sparseness, and the presence of noise (Akrami et al. 2012). One important question is how sparse is the representation. Computationally, it has been suggested that a sparse code will produce an efficient memory encoding, since distinct items are less likely to interfere when represented simultaneously, and it is more likely that a single-layer neural network can learn to generate a target output if the input is represented within a sparse population of neurons (Willshaw and Dayan 1990). On the other hand, a dense code imposes only one pattern at a given time, while superimposing more than one pattern creates decoding difficulties and introduces interference. This is why a dense code limits the number of memories that can be stored, while a sparse code enhances it. However, as we will see, it is not yet clear how the biological network achieves a sparse code.

Currently it is well accepted that activity-dependent synaptic plasticity is a principal mechanism for cell-assembly formation, and consequently for memory consolidation, which is the process through which memory is stabilized. Synaptic plasticity also determines the future dynamics of the neural network associated with memory retrieval and behavioral performance. The idea that reverberatory activity leads to synaptic plasticity may well be applied to both transient and persistent forms of synaptic plasticity, underlying both short-term and long-term memory.

However, it is possible that long-term memory may require long-lasting reverberatory activity or more rounds of it, in a way that enables the coincident activation of different signal transduction pathways to promote long-term synaptic plasticity, but it is also possible that salient signals may determine the fate of one particular representation by inducing a fast achieving plastic event. Neuroscientists know that changes in the synaptic weights occur with different temporal fates, which in turn is determined by the persistence of the synaptic changes and this underlies the duration of the corresponding memory (Dudai 2002).

Many details might be missing and it is not yet clear how the biological network achieves a code that allows efficient memory encoding and retrieval, but today we have substantial evidence suggesting that Hebb's ideas and the theory behind the computational modeling studies inspired by them are in general correct. Meanwhile, it is clear that if we characterized important properties of the neural network at the cellular, synaptic, and molecular levels, the resulting knowledge will

contribute enormously to understand the neural network dynamics, because the integration of these biological features into the computational models can change qualitatively and quantitatively the macroscopic dynamic of the neural network during simulation, and this will give us hints to better understand important details of the biological neural network function.

3 What is Actually Occurring in the Biological Neural Network?

About 20 years after the original proposal of Hebb, the Per Andersen laboratory started a visionary research endeavor that led to the experimental finding of long-term changes in the efficacy of synapses as a consequence of high-frequency stimulation (Lømo 1966). This was later characterized as long-term potentiation or LTP (Bliss and Lømo 1973), and it demonstrated that synapse efficiency could actually change as a result of the activity history of a particular pathway as it was previously proposed by Hebb (1949). This finding attracted considerable attention, and the cellular mechanisms underlying LTP were broadly studied (Abraham and Williams 2003; Lynch 2004; Abraham and Williams 2008). These studies started to uncover a prominent role of glutamate NMDA receptors (Lynch et al. 1988; Daw et al. 1993; Malenka 2003), the relevant dynamic calcium signals (Malenka 1991), and the role of other mechanisms such as retrograde messages (Zorumski and Izumi 1993), kinase activation (Routtenberg 1985), and transcriptional regulation (Pittenger and Kandel 1998). Attention was also directed toward the possible behavioral requirement for LTP; with this in mind, Richard Morris's group decided to block NMDA receptors to impair LTP induction and examine the behavioral performance in the Water Maze task. They demonstrated that impairing LTP in the hippocampus, which is involved in spatial information processing, also affected the animals' behavioral performance in the water maze spatial memory task (Morris et al. 1986). This result was confirmed by other laboratories using other behaviors and analyzing other brain regions, and a wide spectrum of evidence supporting this principle soon accumulated (see Martinez and Derrick 1996). In summary, the studies on LTP have demonstrated that activity-dependent synaptic plasticity is required for memory formation. This confirmed the idea that Hebbian modifications are required in the neural networks to achieve memory storage.

However, the question of whether LTP was actually occurring as a consequence of the animal's experience remained elusive, possibly because some of the details implied in the neural ensemble hypothesis were overlooked, such as sparse coding and connectivity. When electrophysiological recordings were made in discrete regions of the CA1 dendritic tree after the animals learned an inhibitory avoidance task, changes in the efficacy of synapses were detected in some of the dendritic regions recorded (Whitlock et al. 2006), and this happened only in trained, but not

in nontrained animals. This was a clear demonstration that LTP or changes in the efficacy of synapses occurred in a sparse population of inputs after a learning experience, which is completely consistent with the current neural network theory.

It has also been demonstrated that structural synaptic changes occurred as a consequence of the synaptic stimulation that induces LTP (Lynch and Baudry 1987; Markus and Petit 1989; Geinisman et al. 1992) and also as a consequence of behavioral experience (Greenough and Anderson 1991; Rampon et al. 2000; Holtmaat et al. 2008). The idea that structural synaptic changes are the underlying mechanism of neural ensemble stabilization was explicitly proposed in Hebb′s original work (1949). Today, we know that behavioral experience can modify the neuroanatomical structure of the neural network. An early example was that motor skill learning increased the density of synaptic contacts in the cerebellum, as shown by electron microscopy (Greenough and Anderson 1991). Moser et al. (in 1994 and 1997) published several studies in which they measured dendritic spine density in the CA1 dendrites of animals that either were given a complex environment experience that promoted spatial learning or were treated as controls (social caging and single cage controls). They found that spine density in the CA1 basal dendrites (*stratum oriens*) was significantly higher in animals exposed to the complex environment than in controls. Importantly, the animals that experienced the complex environment also performed better than controls in a water maze task (Moser et al. 1997).

A major player in experience-dependent structural plasticity is the neural cell adhesion molecule (NCAM). Furthermore, polysialylation of NCAM which is known to be involved in structural synaptic plasticity (Regan and Fox 1995); enables NCAM to promote the relocation of synaptic contacts, instead of simply maintaining them. Endo-neuraminidase (NE) can cleave the bound of polysialic acid from NCAM (Finne et al. 1983), and the intraparenchymal administration of NE into the hippocampus after acquisition of a water maze task impairs spatial memory consolidation and LTP maintenance (Becker et al. 1996). This evidence suggested that spatial memory consolidation requires the induction of synaptic modifications in the hippocampus, and possibly synaptogenesis. Supporting this idea, an LTP-inducing stimulation produced clear structural synaptic plasticity, visualized with the Timm staining method (Escobar et al. 1997).

With this in mind, a series of experiments intended to test the possibility that spatial water maze training induces synaptic remodeling in the hippocampus, were undertaken. We used the Timm staining method to visualize the mossy fiber buttons in the CA3 dendrites and compared the density of these terminals in the CA3 from animals trained for several days in the Morris water maze task with that in the CA3 from swimming and cage control animals. The results revealed that animals overtrained in the Morris water maze task had a higher density of mossy fiber buttons in the CA3 *stratum oriens* compared to the other groups, including animals trained for fewer days (Ramirez-Amaya et al. 1999). Electron microscopy analysis confirmed this result, revealing a higher density of mossy fiber buttons in the CA3 *stratum oriens* with immature ultrastructural features. The conclusion was that an increase in the density of mossy fiber buttons occurred after Morris water

maze overtraining, indicating that learning-induced mossy fiber synaptogenesis in the CA3 *stratum oriens*. Later, we found that water maze overtraining promoted a memory that lasted for about 30 days, and the animals' performance showed a positive correlation with the density of mossy buttons in the CA3 *stratum oriens*, when all groups were included (Ramirez-Amaya et al. 2001). Interestingly, these structural changes were blocked by the systemic administration of an NMDA antagonist, applied before acquisition but not afterward; this treatment also impaired adequate performance in the water maze task, suggesting that NMDA receptors trigger the structural change, as we will see below. These results have been reproduced by others who found that the structural changes are first detectable several days after the training experience (Rekart et al. 2007), suggesting that synaptogenesis might be established by offline reactivation of the underlying neural network, which is consistent with the theory. However, the structural changes are observed in such an extensive area (Ramirez-Amaya et al. 2001) that they might be related to a change in hippocampal network function as well. Current unpublished observations from our laboratory (Carasatorre et al. 2011), obtained with catFISH and behavioral testing, are showing clear improvements in pattern completion and pattern separation in the hippocampi of animals that showed the structural changes as a consequence of spatial water maze overtraining.

These results could reflect structural changes that are nonspecific or unrelated to memory, raising the important question: Are the CA3 pyramidal cells that respond with activity to the behavioral experience that gave rise to the long-lasting memory, the same cells as those with modified synaptic density in their basal dendrites? We approached this question (Carasatorre and Ramirez-Amaya unpublished observations) by simultaneously staining MAP-2 to visualize the CA3 apical and basal dendrites, synaptophysin to detect the presynaptic buttons, and Arc protein to identify the activated cells. In the image analysis, we segmented the dendrites by characterizing MAP2 staining in the confocal stack, and we distinguished between the activated and nonactivated dendrites. This analysis was done in animals that either underwent a water maze experience 60 min before sacrifice, which was similar to their training for 5 days with a submerged platform; other groups contained animals that were pretrained as swimming controls and tested as such, animals that experienced the water maze task before sacrifice but instead of being previously trained they remained undisturbed in their home cage. Finally, one last group of animals did not receive any behavioral treatment at all and were therefore considered as cage controls. At the moment of writing this chapter, preliminary results are revealing an 8-fold increase in synaptophysin density that appears to be more pronounced in the MAP-2-Arc positive dendrites of water maze-treated animals. Interestingly, however, a significant 2- to 3-fold increase was also found in the nonactivated dendrites (nonArc positive MAP-2 only dendrites) of water maze-treated animals. Therefore, most of the synaptophysin and possibly the synapse density increase occur in the activated dendrites, but some might be occurring in the nonbehavioral specific activated dendrites. So far this finding supports the idea that structural synaptic plasticity is a persistent type of

change in the neural synapses underlying long-term memory formation (Bailey and Kandel 1993), and it may well be one of the underlying mechanism that explains long-term memory formation (Bailey and Kandel 2008). Other studies had found more subtle structural synaptic changes as a consequence of behavioral experience (Rusakov et al. 1997), so it is likely that the structural changes seen after behavioral experience (Ramirez-Amaya et al. 1999, 2001) have important implications for both network function and memory storage.

Nevertheless, further work is needed to distinguish which structural synaptic changes account for the fine tuning of the network function and which are truly related to the stabilization of the acquired representations and their maintenance in memory. It is important to note that the persistent forms of neural plasticity underlying long-term memory may also include homeostatic synaptic plasticity (Perez-Otano and Ehlers 2005; Shepherd et al. 2006; Rabinowitch and Segev 2008; Peebles et al. 2010; Sajikumar and Korte 2011) and changes in the intrinsic properties of the neuron activity (Marder et al. 1996; Barkai 2005; Narayanan and Johnston 2007). However, it is not clear yet whether this or other types of plasticity should occur in all or some of the inputs involved in the circuit or the neural units that were recruited. Since the molecular signals underlying plasticity are both deterministic and probabilistic, one may think that plasticity may have different strengths and temporal fates in each input and the neural units involved.

The theory also states that the neuronal population, as opposed to single neurons, will reliably encode the processed information; although single neurons process features from the stimulus, their activity is very noisy and not reliable enough to support storage and retrieval. In the 1980s, Georgopoulos and his group developed the population vector analysis for multiple-unit electrophysiological recording of motor cortical neurons of macaques performing an arm direction task (Georgopoulos et al. 1986; Kettner et al. 1988). They successfully demonstrated that groups of neurons encode motor information, in a way that the analysis of a neuronal population yielded enough information that could be used to predict the actual motor behavior of the primate (Georgopoulos et al. 1986; Kettner et al. 1988). A similar analysis done on the recorded activity of a population of hippocampal place cells, predicted the actual location of the animal during the exploration of a spatial environment (Wilson and McNaughton 1993); in this case, the larger the population of hippocampal neurons recorded, the more reliable the prediction of the animal′s location became. In fact, today this notion is being exploited to develop motorized limbs for rehabilitation of subjects who have lost an arm or a leg. In this case, population recordings of the subject's own neural activity are used to control the limb movement (Velliste et al. 2008; Wang et al. 2010). Together, this evidence demonstrates that population coding is the mechanism of information processing in the brain, as suggested by the theory. The analysis methods currently being developed include promising new strategies to electrophysiologically detect functional ensembles of neurons (Tsien 2007; Chen et al. 2009).

Another prediction of the theory is that the neural units recruited during the acquisition of new information, or at least some of them, should be the ones

activated during retrieval. Over short time intervals or moderately long-term memories (a few days), compelling evidence supporting this view was provided by electrophysiological recordings of place cells in the hippocampus. Place cells are hippocampal pyramidal cells that show a clear increase in the firing rate when the animal is located in a particular place of the environment (O'Keefe 1976; Nadel and Eichenbaum 1999; Jeffery 2007; Moser and Moser 2008). This means that the firing rate of an individual neuron increases in one particular place, and this response is repeated most of the time when the animal travels through the *place field* of the cell. The area and placement of the place field, i.e., where the cell responds, remains in that location throughout daily recordings in the same environment (Thompson and Best 1990; Kentros et al. 2004). Also, with the catFISH analysis, in which we detect genes that are expressed immediately after synaptic neural activity and the location of the label within the cell, we can distinguish cells recently activated (5 min with two Arc foci) from those activated earlier (30 min, with Arc in the cytoplasm). It has been observed that the majority of the cells that are activated with one environment are activated again with exploration experience in the same environment (Guzowski et al. 1999). This result demonstrates that of the cells activated during acquisition, ~ 80 % are reactivated during retrieval. But for longer time intervals, the evidence is not quite clear. Electrophysiological recordings do not provide the best neuroanatomical resolution and the implanted electrodes may produce necrosis. Other methods to characterize the neuroanatomical patterns of activity with accurate cellular resolution might provide more accurate answers.

With an elegant transgenic mouse strategy that used the promoter of the immediate early gene (IEG) *cFos* attached to a tTA sequence that can activate a constitutive expressed tag (LacZ) when the system is activated in the absence of doxycycline, which maintains the system off. With this system Leon Reijmers group were able to label neurons, activated during the acquisition of a fear conditioning task, for a prolonged period of time after the experience. Three days later, the animals were exposed again to the same task and the acquisition-labeled neurons were compared with those activated during retrieval by detecting Zif268 expression. Here it was demonstrated that the neurons activated during retrieval were a subpopulation of the neurons activated during acquisition. However, the proportion of neurons that were recruited during retrieval was only about 12 % from those activated during acquisition (Reijmers et al. 2007). One source of error comes from the fact that the animals need to stop taking doxycclyne about 24 h before the acquisition of the task, so some of the neurons tag with LacZ and may have been activated by other stimuli. More precise long-term labeling systems need to be developed to accurately determine how many cells recruited during acquisition are also recruited during long-term retrieval. If the idea suggesting that only a fraction of the originally recruited neurons are the ones that are recruited during retrieval is right, this will indicate that this subpopulation of neurons might be the ones that underwent synaptic plasticity, a notion that was also suggested by data obtained from offline reactivation.

According to the theory, the neuronal ensemble should also be able to reactivate offline or in the absence of further stimulation. This has also been experimentally demonstrated. Wilson and McNaughton (1994) recorded a population of neurons in hippocampal area CA1 and found that cells that were active together during spatial exploration also tended to fire together during the sleep period that immediately followed the spatial experience. In this analysis, pairs of neurons that were classified as being 'correlated' or 'uncorrelated' maintained this classification during the reactivation period, suggesting that during sleep, the network replays the state imposed during the experience. We now know that place cell reactivation that occurs during sleep can also be observed during periods of wakeful rest (Karlsson and Frank 2009; Carr et al. 2011) or pauses during spatial behavior (Davidson et al. 2009), and that this reactivation produces a sequential replay of the activity patterns acquired during the initial experience (Karlsson and Frank 2009; Carr et al. 2011). The awake state offline reactivation is believed to reflect trajectories through either the current environment or previously visited ones that are spatially remote (Davidson et al. 2009; Karlsson and Frank 2009; Carr et al. 2011). This repetition of acquired activity patterns on a compressed time scale is suggested to promote memory formation in distributed neural circuits.

Using electrophysiological recording, it is possible to characterize in detail the sequential activity of neural ensembles recruited during experience that are later reactivated in an offline period. However, the number of cells included in the analysis of these electrophysiological studies is around one hundred units, and the anatomical extent of the study is limited to two or three different brain regions. Thus, in order to extend the anatomical reach of the analysis, other methods have been developed. Such a method can be the detection of immediate early genes (IEGs) like *Arc*, which was used to develop catFISH (Guzowski et al. 1999). Using a modified version of it, we were interested in determining whether or not the translation of Arc was limiting the number of plastic cells; since the size of the Arc-expressing ensemble is quite large ($\sim$45 % of total neurons in CA1 and Cortex) and since Arc protein is known to be involved in long-term synaptic plasticity, some modulation in the size of the population in which synaptic plasticity is achieved would be expected. We found that Arc translation was not the limiting factor, since the proportion of Arc mRNA and Arc protein positive cells was very similar; however, 8 h after the original experience, a subpopulation of about 50 % of the neurons activated during acquisition, reactivated the expression of Arc. More than 80 % of the cells that show Arc protein expression at 8 and 24 h also respond to the second exploration by expressing *Arc* mRNA (Ramirez-Amaya et al. 2005). We interpret this finding as a highly specific, offline reactivation of Arc expression in a subset of the originally activated cells. Interestingly, we note that only about 50 % of the originally activated neurons underwent reactivation and, although 20 % of CA1 cells still represent a big population, we hypothesize that this could be a selection mechanism that promotes a sparse code for memory encoding. This evidence is also in line with the idea that a subpopulation of the originally activated neurons is likely to elicit synaptic plasticity and suggests that

not all neurons recruited during the original acquisition of information undergo synaptic plasticity.

By visualizing the expression of Arc-GFP in a Tg mouse (Arc promotor linked to green fluorescent protein), using two-photon live imaging in the living mouse brain, it was possible to make the astonishing observation that, after repeated stimulation with a visual stimulus of one specific orientation, the size of the Arc-expressing ensemble in the visual cortex gradually decreased over a series of daily exposures (Wang et al. 2006). The final proportion of cells activated by the repeated experience was again about 50 % of the originally activated ensemble. Moreover, they also found that the subset of cells that had kept firing together after several consecutive days had a higher likelihood of continuing to fire together, whereas the cells that had stopped firing were less likely to be part of the recruited ensemble. But remember that here they were identifying the active neurons by the use of Arc reporter (Wang et al. 2006). This decrease in the active neurons has not been observed with electrophysiology, possibly we may need more anatomical accuracy with this method; however it is also clear that for IEGs expression, in the hippocampus, after several daily exploration experiences, the number of neural units activated remain the same, which is not observed after massive exploration that occur in one day (Guzowski et al. 2006). So if the system manages to select the plastic neural units, the question is why and how it keeps recruiting neural populations of similar sizes after long-term experiences.

Taken together, IEG detection studies in which the neuroanatomical activity patterns, as described above, were characterized, suggest that during either offline or online reactivation, the decrease in the number of active neurons may be a mechanism through which the system "fine-tunes" the neural representation and selects the units that will undergo persistent types of synaptic plasticity and be stored in long-term memory (Ramirez-Amaya et al. 2005; Wang et al. 2006). This interpretation is gaining support from preliminary results from our lab showing that the neurons engaged in offline reactivation are more likely to coactivate CREB (Morales and Ramirez-Amaya 2010). An important idea behind this is that synergic activation of different signal transduction pathways may modulate or determine the plastic fate of a subset of the neural units that were originally activated. According to our current estimates, the size of this subset of "plastic" neurons may be of about 35–40 % of the originally activated network, and estimates match between studies (Ramirez-Amaya et al. 2005; Wang et al. 2006). Since the size of the ensemble during long-term retrieval is likely to remain the same, this suggest that we may have two populations of neurons that reactivate the activity pattern: 35 % of these are Hebbian neurons (those that achieved synaptic plasticity), and 65 % are nonHebbian neurons (the ones that did not achieve it). Since the size of the activated ensemble in each experience, acquisition, and retrieval, is the same, the question is, whether the 65 % nonHebbian, reactivated neurons were recruited stochastically or tend to be part of the originally activated population; the answer is more likely to be the latter, since electrophysiological studies tend to record the same neurons with the same place field over several days of recording (Davidson et al. 2009; Karlsson and Frank 2009; Carr et al. 2011).

Obviously, accurate counts are needed and can be obtained with methods such as that of Reijmers, which allow us to perform experiments with long-term neural activity labels and determine with precision the number of units that were actually active in both experiences. But if their synaptic weights do not change, why and how do the neurons manage to keep firing together? is this is a feature of the attractor network? and how reliably, and for how long are the same neural units selected? And again, we need to know the exact size of the Hebbian neuron population that undergoes long-term plastic changes underlying long-term memory.

Last but not least, an interesting story is currently unfolding in the hippocampus dentate gyrus; it has been observed that the dentate gyrus uses an impressively clear sparse code that appears instantly as the initial response to a spatial exploration experience (Chawla et al. 2005; Ramirez-Amaya et al. 2005), where the proportion of neural units activated is around 2 % (Chawla et al. 2005; Ramirez-Amaya et al. 2005). This is a remarkable example of a neural network using sparse coding to orthogonalize information that eventually reaches the pyramidal cell dendrites of CA3. However, it has been found that when exposing animals to five different environments in one long session, instead of activating 2 % of neurons multiplied by the number of explored environments, the proportion of activated cells is only about 3 % of the total population of neurons. With electrophysiological recordings, it was observed that the same cells expressing place fields in one environment, also express them in other environments (Alme et al. 2010). This suggests that instead of using different neural units to encode different information, the neural networks in the DG may use different activity pattern sequences in the same neurons. This contrasts with the clear independence of the ensembles recruited with two different environment experiences in the CAs of the hippocampal and pyramidal neocortical cells. The evidence then suggests that plasticity in DG neurons is highly likely to be achieved in the initially recruited ensemble during the acquisition of novel spatial information by a mechanism of sustained Arc transcription (Angulo et al. 2009; Ramírez-Amaya et al. 2005).

In contrast to the CA regions, in the DG network, different elements of spatial information appear to be represented in different sequences of activity in the same cells, the size of the ensemble is smaller (about 2 % of the total population of neurons) and plastic cells are selected immediately upon initial recruitment, through a mechanism related to sustained transcription of *Arc* mRNA (Angulo et al. 2009). However, and possibly counter intuitively, the DG units that participate in a given representation may change gradually over time (Alme et al. 2010; Sandoval et al. 2011) and possibly with experiences. These results lead to the granular cell early retirement hypothesis. These interesting cellular features of the DG network suggest that neural networks may present at least two different ways to organize the acquired patterns of activity: (a) by using different activity pattern sequences with the same neural units or (b) by recruiting different neural units which, as a group, produce one particular activity pattern. This notion should be tested in computational models to determine whether or not it contributes to

facilitate neural networks dynamics and to what extent it may translate into a revised description of the theory.

All in all, there is compelling evidence that plasticity in the communication between neurons is indeed involved in the neural mechanisms that lead to memory formation, but many details still need to be added or adjusted. For example, a precise characterization of the number and type of neural units involved in information processing and storage, i.e., those undergoing synaptic plasticity is urgently needed. This will enable us to test the algorithms used in the neural network models. A detailed characterization of the cellular, synaptic, and molecular changes underlying long-term memory formation is also required to generate biologically realistic neural network models.

In the following pages, we will present a general review of the molecular events related to long-term synaptic plasticity, which is intended to provide a clearer understanding of the underlying cell signaling principles of long-term memory formation.

4 Triggering Signals

Synaptic plasticity underlying long-term memory formation may take several forms. Here, we will consider that the plastic changes related to long-term memory are those that persist over time, such as structural synaptic plasticity and homeostatic synaptic plasticity. These persistent forms of synaptic plasticity underlying ensemble stabilization can be driven by the activation of one or several neurotransmitter receptors.

The most important excitatory neurotransmitter in the central nervous system (CNS) is L-glutamate, and it is well accepted that changes in the efficacy of glutamatergic synapses depend on changes in the density and features of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and NMDA receptors. Glutamatergic transmission plays an important role in the stabilization of synaptic contacts (Rebola et al. 2010; McKinney 2010). It is well established that LTP induction promotes changes in the synaptic structure (Desmond and Levy 1983; Escobar et al. 1997); for example, inhibition of LTP with an NMDA receptor antagonist (D(-)-2-amino-5 phosphonovaleric acid; AP5) prevents these structural changes (Toni et al. 1999). Likewise, we found that water maze overtraining induces changes in the distribution of mossy fiber boutons in the CA3 region of the hippocampus (Ramirez-Amaya et al. 1999), and these changes were blocked by pretreating animals with the NMDA receptor antagonist MK801 (Ramirez-Amaya et al. 2001). Other studies show increased spine density in the hippocampus which was observed 24 h after trace eyeblink conditioning, and again, this change was blocked by NMDA receptor antagonists (Leuner et al. 2003). Together, these data indicate that glutamatergic transmission is a very important first step in the mechanism(s) underlying structural synaptic plasticity.

Remarkably, AMPA receptors are responsible for the generation of excitatory post-synaptic potential (EPSP) and are the triggers of synapse efficacy changes,

such as LTP and LTD. But of great relevance are the NMDA receptors, which regulate the changes in the efficacy of synaptic transmission and have long been known to be involved in learning and memory. These receptors participate in the fine tuning of neural networks by modulating the generation of synaptic contacts as well as strengthening, weakening, and pruning them (Malenka and Nicoll 1993; Huang and Pallas 2001; Lüthi et al. 2001; Adesnik et al. 2008).

NMDA receptors are tetramers composed of two different subunit types, NR1 and NR2. These subunits have an N-terminal extracellular domain, three transmembrane regions, and an intracellular C-terminal domain that interacts with several proteins and can be phosphorylated, regulating receptor trafficking and function (Salter and Kalia 2004; Lau and Zukin 2007; Groc et al. 2009). Different pharmacological and biophysical properties of the multiple NMDAR subtypes are determined by the type of NR2 subunit (NR2A to NR2D) incorporated into the heteromeric NR1/NR2 complex. The NR2 subunit is the crucial regulatory unit of NMDARs, since it contains the glutamate-binding site, the glycine-binding site, and affinity site for Mg^{2+} blocking of the channel (Traynelis et al. 2010). This subunit modulates the affinity for glutamate and mediates glycine binding, and it also determines the strength of Mg^{2+} binding, which blocks NMDA channel function and modulates Ca^{2+} conductance (Cull-Candy and Leszkiewicz 2004; Paoletti and Neyton 2007). The NR2 subtypes NR2A and NR2B have very similar amino acid sequences, but they differ in their C-terminal ends (Ishii et al. 1993). These NMDA receptor subtypes significantly influence synapse morphology; it has been demonstrated for example that the early expression of NR2A in organotypic, hippocampal slice cultures reduces the number of synapses and the volume and dynamics of dendritic spines (Gambrill and Barria 2011). In contrast, NR2B overexpression apparently does not affect the normal density of synapses (Gambrill and Barria 2011), but it does increase spine motility, adding and retracting spines at a higher rate (Gambrill and Barria 2011). Similarly, overexpression of NR2B increases the motility of filopodia (Gambrill and Barria 2011), whose role is to establish synaptic connections (Dailey and Smith 1996; Portera-Cailliau et al. 2003; Holtmaat et al. 2006). These results show that the balance of NR2A and NR2B in the synapse strongly affects the number of synapses formed, suggesting that NR2A prevents the process of synaptogenesis, while NR2B triggers spinogenesis and synaptogenesis by increasing the rate of structural plasticity, enabling the neuron to find and establish efficient synaptic contacts. The proposal that NMDARs are fundamental for synaptic and spine stabilization (Alvarez et al. 2007; Akashi et al. 2009) is supported by experimental evidence showing distinct roles for the C-termini of the two NR2 subunits in forming and stabilizing synapses. Congruently, NR2B-knockout mice exhibit a reduction in G-actin and in the density of dendritic spines (Gambrill and Barria 2011). The difference between NR2A and NR2B may be related to their ability to interact with Ca^{2+}/calmodulin-dependent kinase II (CaMKII) which, as we will see in the next section, is of great importance for structural synaptic plasticity. The ability of NR2B to bind CaMKII depends on its C-terminal sequence, which is considered to be critical for the regulation of synaptogenesis (Gambrill and Barria 2011); in contrast, the C-terminal sequence of NR2A does not bind CaMKII and when it replaces NR2B,

CaMKII-mediated synaptic potentiation is reduced (Gambrill and Barria 2011). Cleary this shows how the NMDA receptor subunit composition is crucial for synaptogenesis and synapse stabilization.

Other interesting evidence shows that stimulation of AMPA and NMDA receptors reduces the motility of spines during development (Fischer et al. 2000), suggesting that the stabilization of contacts might be driven by glutamate receptor-mediated activity. This may also be true for synapse formation in the adult brain during structural plasticity related to long-term memory (Ramirez-Amaya et al. 2001; Rekart 2007). Actin filaments are thought to be crucial for both the formation of dendritic spines during development and their structural plasticity at mature synapses (Fischer et al. 2000; Matus et al. 2000), and the activation of NMDA receptors is important for integrin-dependent actin reorganization (Shi and Ethell 2006), which is known to induce the elongation of existing dendritic spines and to promote the formation of new filopodia (Shi and Ethell 2006). Time-lapse imaging of fluorescent glutamate receptors, demonstrated that NMDA and AMPA receptors are present in motile filopodia before and during synaptogenesis (Washbourne et al. 2002), and it was demonstrated that the activation of these glutamatergic receptors modulates the motility of filopodia (Chang and De Camilli 2001), supporting the idea that the formation of new synapses may depend on glutamatergic activity.

Another feature of glutamatergic transmission is that the number of AMPA and NMDA receptors in the postsynaptic membrane depends on the activity history of the synapse (Lüscher et al. 1999; Shi et al. 1999; Lan et al. 2001; Crump et al. 2001). The regulation of glutamate receptor density in the postsynaptic membrane has also been implicated in a special form of synaptic plasticity known as synaptic scaling (Perez-Otano and Ehlers 2005; Wierenga et al. 2005; Andrasfalvy and Magee 2001). This is a homeostatic regulation of the synapse, in which the total synaptic strength is modulated in order to adjust to recent changes in the efficiency of the synapse, and it is required to stabilize the plastic changes in the neural network (Turrigiano et al. 1998; Abbott and Nelson 2000; London and Segev 2001; Perez-Otano and Ehlers 2005). Thus, the mechanisms that regulate the aggregation, endocytosis, and trafficking of glutamate receptors in the postsynaptic membrane may contribute to this persistent form of synaptic plasticity.

Although memory formation clearly requires the early involvement of NMDA, AMPA, and metabotropic glutamate receptors, it is also regulated by cholinergic and GABAergic transmission (Izquierdo et al. 1999). An interaction between cholinergic and glutamate receptors has been postulated, since their activity converges, as demonstrated by studies of the multiple signal transduction pathways mediated by these receptors (Ferreira et al. 2002; Woolf 1998). Possibly, different signal transduction cascades of fast (e.g., glutamatergic) and modulatory (e.g., cholinergic) neurotransmission are both necessary for long-term synaptic plasticity and may converge in a given neuron (Rosenblum et al. 2000). Moreover, it has been suggested that this convergent signaling may promote morphological changes in the neurons where it occurs (Woolf 1996). These ideas led to the hypothesis of a cholinergic regulation of long-term synaptic plasticity, which suggests that cholinoceptive cells may undergo changes in their dendritic structure as a result of

Ach (acetylcholine) receptor activation, which is proposed to induce the degradation of MAP-2 structures (Woolf 1996). Such structural changes may occur during memory consolidation and may be responsible for long-term memory storage. It has been shown that cholinergic receptors mediate NGF-induced excitatory synaptogenesis (Woodin et al. 2002), lending support to the idea that ACh is one of the molecular signals leading to structural synaptic plasticity. Further support comes from experiments in rats in which postnatal lesions of the nucleus basalis magnocellularis that produced a robust cholinergic deafferentation in the cortex, altered the differentiation of cortical neurons and synaptic connectivity that persists into maturity, and contributed to altered cognitive behavior (Hohmann 2003). In the honey bee brain, treatment with pilocarpine, a muscarinic agonist, induced an increase in the volume of the neuropil similar to that observed after foraging behavioral experience (Ismail et al. 2006). Recently, it has been demonstrated that the activation of muscarinic receptors in CA1 pyramidal neurons induced the emergence of fine filopodia from spine heads (Schätzle et al. 2011). This is one of the most direct pieces of evidence for ACh participation in structural synaptic plasticity.

Another neurotransmitter that may be involved in persistent forms of synaptic plasticity is dopamine; for example, it has been demonstrated that overexpression of D2 dopamine receptors decreases the complexity and length of the dendritic arbors of the neurons (Cazorla et al. 2012), and that these changes can be reversed by restoring D2 dopamine receptors to normal density (Cazorla et al. 2012).

Many other neurotransmitters may also trigger or modulate persistent forms of synaptic plasticity (Imai et al. 2004); possibly, a synergistic action between various different systems is required to induce long-lasting forms of synaptic plasticity. Nevertheless, these initial signals may converge in some common cellular events, such as the influx of calcium and the activation of the kinase-phosphatase system, among others. The increase in Ca^{2+} conductance mediated through NMDA receptors can trigger the activity of several kinases, such as CaMKII, PKA, PKC, and MAPK (Kerchner and Nicoll 2008; Newpher and Ehlers 2009).

5 Downstream Signals

Once the receptors are activated, several downstream signals modulate the final cellular events that persistently stabilize the synaptic connections. As we mentioned earlier, probably the most prominent signal associated with synaptic plasticity is calcium. In general terms, in dendritic spines, elevation of Ca^{2+} activates numerous signaling proteins including protein kinase C (PKC), Ca^{2+}/calmodulin-dependent kinase II (CaMKII), and small GTPase proteins such as Ras and Rho (Kennedy et al. 2006). All of these proteins are important regulators of long-term synaptic plasticity, and they promote different cellular processes including actin polymerization and depolymerization, trafficking of proteins, and exocytosis and

endocytosis of glutamate receptors (Kennedy and Ehlers 2006; Hotulainen and Hoogenraad 2010).

Decidedly, the principal calcium signal transducer is CaMKII. After synaptic activation, the influx of Ca^{2+} into the cell, either through ligand- and voltage-gated calcium channels or from internal reservoirs, results in a complex set of transitory and oscillatory signals. These complex signals require a molecular device to transform them into a more stable and durable message; such a device should be capable of activating the intracellular cascades involved in stabilizing synaptic plasticity. CaMKII is a ubiquitous Ser/Thr protein kinase with broad specificity, and it is very abundant in the CNS. This enzyme is highly concentrated in the postsynaptic density where it is considered to be an important Ca^{2+} "detector" (Lisman and McIntyre 2001).

The unique regulatory properties of CaMKII make it an ideal "interpreter" of the diversity of Ca^{2+} signals. Evidence has shown that CaMKII can interpret the message coded in the amplitude and duration of individual Ca^{2+} spikes and translate it into distinct amounts of long-lasting, Ca^{2+}-independent kinase activity (DeKoninck and Schulman 1998). CaMKII is composed of subunits that combine into a dodecamer, in which each subunit work as a serine–threonine kinase (Rosenberg et al. 2005). In its nonactivated state, CaMKII is autoinhibited or blocked, but it becomes activated when it interacts with calcium/calmodulin (Ca^{2+}/CaM) complexes (Lisman et al. 2002). Active CaMKII phosphorylates many proteins, and it also displays an important autophosphorylation activity. When phosphorylated, CaMKII remains active even after the dissociation of Ca^{2+}/CaM. Thus, autophosphorylation generates a constitutive active form of CaMKII able to translate a transient Ca^{2+} signal into a persistent, Ca^{2+}-independent signal (Cammarota et al. 2002). The ability of CaMKII to maintain its phosphorylating activity for a prolonged period of time through autophosphorylation (Lisman et al. 2002) represents an important way to sustain the effects of Ca^{2+} signaling. Since this is a mechanism that can persist for up to several minutes, it may be of great relevance for the consolidation of long-term synaptic plasticity and memory formation (Giese et al. 1998).

The active form of CaMKII is found in the postsynaptic density (Yoshimura et al. 1996), where it interacts with a variety of molecules important for the structure and function of the postsynapse. These include PSD-95 (Yoshimura et al. 2000), densin-180 (Strack et al. 2000; Walikonis et al. 2001), F-actin (Allison et al. 2000), and particularly the NMDA glutamate receptor (Gardoni et al. 1998). The interaction with the NMDA receptor is very important since it increases CaMKII autophosphorylation activity and its ability to become hyperphosphorylated (Bayer et al. 2001). Hyperphosphorylation of CaMKII also enhances AMPA receptor conductance by causing the phosphorylation of AMPA receptors (Derkach et al. 1999) and it can extend the period of CaMKII activation by saturating local phosphatases, preventing dephosphorylation (Lisman and Zhabotinsky 2001). Finally, hyperphosphorylation of CaMKII may increase the interaction between NMDA and AMPA receptors (Lisman and Zhabotinsky 2001). This interaction may in turn induce the insertion of AMPA receptors into 'silent' synaptic sites that already contain NMDA receptors (Hayachi et al. 2000). All these functional

properties of CaMKII are crucial for its involvement in the persistent forms of synaptic plasticity.

It has been proposed that CaMKII works as a memory switch for Ca^{2+} signaling, alternating between a stable 'on' state and a transient 'off' state (Lisman and McIntyre 2001). Bayer and colleagues suggest that CaMKII presents an initially reversible state that is dependent on Ca^{2+}/CaM-binding at the substrate-binding site ("S-site"); and later CaMKII assumes a more stable state in which a persistent interaction with its T286-binding site ("T-site") enables the Ca^{2+}/CaM-independent activity of the kinase. This may indeed be the most prominent feature of CaMKII (Bayer et al. 2006).

Various pharmacological inhibitors of CaMKII have been developed, and they consistently have been found to inhibit the induction of LTP (Malinow et al. 1989; Ito et al. 1991; Hvalby et al. 1994; Otmakhov et al. 1997; Buard et al. 2010; Hayachi et al. 2000; Watanabe et al. 1996; Link et al. 1995; Guzowski et al. 1999). On the other hand, when the constitutively active form of CaMKII is injected or overexpressed in neurons, synaptic strength is potentiated (Lledo et al. 1995; Hayachi et al. 2000). Also, CaMKII activity is required for the maintenance phase of LTP (Wang and Kelly 1995). Importantly, we know that both the induction of LTP and sensory stimulation promote a rapid growth of dendritic filopodia and the formation of dendritic spines and new synapses (Escobar et al. 1997; Toni et al. 1999; Engert and Bonhoeffer 1999). In a beautiful study using hippocampal slice cultures (Jourdain et al. 2003), the intracellular administration of the autophosphorylated form of CaMKII reproduced the effects of LTP, including filopodia growth and spine formation. The inhibition of phosphatases, thereby maintaining CaMKII activity, or the application of Ca^{2+}/CaM in neurons produced the same effect. Furthermore, blocking CaMKII activity prevented LTP, filopodia growth, and spine formation (Jourdain et al. 2003).

Other inhibitors of CaMKII such as KN62 and KN93 (Wayman et al. 2008) can also block structural synaptic plasticity induced by 2-photon glutamate uncaging (Matsuzaki et al. 2004; Harvey et al. 2008; Steiner et al. 2008; Lee et al. 2009). Interestingly, application of the CaMKII inhibitor KN62, or the NMDAR antagonist AP5, prevents the glutamate-induced, long-term enlargement of the dendritic spines, indicating that activity-dependent spine growth requires both CaMKII activity and NMDAR activation (O'Keefe and Nadel 1978).

Of the different CaMKII genes that have been described, only CaMKII-β, but not CaMKII-α, has strong morphogenic activity which regulates dendritic growth, filopodia extension, and synapse formation in cell cultures (Fink et al. 2003). For example, it has been shown that reducing the amount of the CaMKII-β subunit, but not CaMKII-α, clearly changes the shape of spines by turning them into filopodia-like structures (Okamoto et al. 2007). This suggests that CaMKII-β may regulate structural plasticity of spines by directly regulating actin dynamics (Cingolani and Goda 2008; Dillon and Goda 2005; Sekino et al. 2007). Two forms of actin, the monomeric globular form (G-actin) and the filamentous form (F-actin), coexist in a dynamic equilibrium that is modulated by proteins that either promote actin depolymerization (such as cofilin) or polymerization (such as profilin) (Okamoto

et al. 2009). CaMKII heterooligomers may also bind to different actin filaments through multiple β-subunits, since F-actin forms thick-bundled structures in vitro in the presence of the CaMKIIβ (O'Leary et al. 2006; Okamoto et al. 2007; Sanabria et al. 2009). It was found that CaMKII-β stabilizes the actin cytoskeleton in spines and also that phosphorylation of CaMKII reduces the binding activity of β-subunits (Okamoto et al. 2007), which suggests that the ability of CaMKII to stabilize the actin cytoskeleton is regulated by synaptic activity.

However, it is important to note that the kinase activity of CaMKII is also essential for structural plasticity (Matsuzaki et al. 2004; Okamoto et al. 2004). CaMKII initiates multiple signaling pathways by phosphorylating proteins such as GluR1 or PSD-95 and by activating the Rho family of small GTPases that indirectly through downstream signals regulates the G-actin/F-actin equilibrium. However, the main target of its kinase activity may actually be CaMKII itself (Shen et al. 1998).

The phosphorylation status of CaMKII regulates its ability to bind directly to F-actin. In basal conditions, actin filaments are bundled by CaMKII through its β-subunit, thereby maintaining the stability of spine structure. The activation of NMDA receptors and the resultant Ca^{2+} influx trigger CaMKII kinase activity, inducing its autophosphorylation and detaching it from actin filaments. This mobilized CaMKII can now diffuse freely to phosphorylate other targets and, at the same time, the actin cytoskeleton is no longer stabilized and it can remodel and change the structure of its spines. When eventually, CaMKII returns to the unphosphorylated basal state, phosphatases prevail over the signaling cascades and dephosphorylate their target proteins, the nonphosphorylated CaMKII then reassociates with the actin filaments, promoting their aggregation and consolidating the spine remodeling. This structural remodeling has several important consequences for the efficiency of the synapse, since it provides a new structure that may increase the number of sites available to hold postsynaptic proteins, which may help sustain the increased synaptic efficiency after LTP.

As we can notice, the participation of CaMKII in structural synaptic plasticity is consistent with the idea that CaMKII has important roles in long-term memory. Congruently, training in the Morris water maze task induces the activation of CaMKII in the hippocampus and interestingly, retention performance in this spatial memory task shows a positive correlation with the levels of CaMKII activity (Tan and Liang 1996). Although animals with heterozygous mutations of CaMKII have normal memory retention for contextual fear and the water maze task 1–3 days after training, they are amnesic when tested 10–50 days later, suggesting that long-term memory, but not short-term memory, depends on CaMKII (Frankland et al. 2001).

In summary, CaMKII is an essential multifunctional macromolecule for maintaining, remodeling, and stabilizing the synaptic structure, and it can also modulate the activity of other plasticity-related proteins that contribute to the long-term changes of synaptic efficacy. Thus, CaMKII plays a dual role in activity-dependent synaptic plasticity: a signaling role during transient Ca^{2+} influx and a structural role during the following basal state, linking functional and structural plasticity. Another

pathway implicated in the persistent forms of synaptic plasticity is the ERK1/2-MAPK cascade, which is also a major target of calcium signaling. It is activated by glutamate receptors such as the NMDA and AMPA receptors, trophic factors such as BDNF, and by the neuromodulators acetylcholine and dopamine, among others (Wiegert and Bading 2011). This pathway controls many signaling substrates in both the nucleus and the cytoplasm. Within the cytoplasm, calcium ions, PKA, PKC directly or indirectly activate small GTPases such as Rap-1 or Ras. Both of these GTPases phosphorylate Raf proteins which, in turn, phosphorylate MEK1/2, which subsequently phosphorylates ERK1/2.

When phosphorylated, ERK1/2 also phosphorylates ribosomal protein S6 kinase 2 (RSK2) (Xing et al. 1996), and it modulates AMPA receptor trafficking (Derkach et al. 2007; Kim et al. 2005; Patterson et al. 2010) and consequently, the density of postsynaptic AMPA receptors, thereby triggering the outgrowth and stabilization of dendritic spines (Dinev et al. 2001; Huang et al. 2007; Westmark et al. 2010). Thus, this pathway participates in remodeling the postsynaptic cytoskeleton which, as mentioned before, is important for the persistent forms of synaptic plasticity and long-term memory formation.

Phosphorylated ERK 1/2 may also be important to explain synaptic tagging, which is how activity-dependent synaptic plasticity occurs at specific synaptic sites, since it is known to regulate activity-dependent local translation of mRNAs (Wiegert and Bading 2011) as well as their targeting to the activate spines.

Another way in which this system modulates plasticity is that phosphorylated ERK1 and ERK2 can enter the nucleus where they regulate gene transcription (Davis et al. 2000; Rosenblum et al. 2002; Guzowski et al. 2000; Ying et al. 2002; Waltereit et al. 2001). ERK1/2 also phosphorylates specific transcription factors like Elk-1, as well as mitogen- and stress-activated kinase 1 (MSK1), which is involved in CREB phosphorylation (Wiegert and Bading 2011); CREB, in turn, regulates long-lasting plasticity in several ways, as we will discuss below. ERK1/2 activity can also promote chromatin remodeling through the phosphorylation of histone H3 (Gille et al. 1995; Treisman 1992; Brami-Cherrier et al. 2007, 2009; Wiegert et al. 2007). This last mechanism regulates gene transcription by enabling specialized enzyme complexes to bind to uncondensed chromatin, making gene promoters accessible to the transcription machinery. Chromatin remodeling and structural plasticity of the nuclear envelope provide the neuron with large-scale mechanisms to adapt the gene expression programs to changes in its activity state. These forms of ERK1/2-dependent regulation of gene expression in response to neuronal stimulation occur in several physiological processes in the brain. In particular, the nucleosomal response is involved in LTP regulation as well as in stress-related memory (Chandramohan et al. 2008; Reul et al. 2009) and contextual fear conditioning (Mifsud et al. 2011). Other transcription factors important for synaptic plasticity that are regulated by ERK1/2 are Zif268, BDNF, Arc, c-Fos, and c-Jun (Davis et al. 2000; Rosenblum et al. 2002; Guzowsk et al. Guzowski et al. 2000; Ying et al. 2002; Waltereit et al. 2001). The promoters of many of these genes contain regulatory elements controlled by kinases other than ERK1/2. In these cases, nuclear ERK1/2 signaling is necessary, but not sufficient, to elicit

robust and long-lasting, CREB-dependent gene transcription (Chawla et al. 1998; Kornhauser et al. 2002).

Several routes exist by which calcium can modulate the transcription machinery. Thus, a variety of transcription factors are regulated by calcium signaling either inside or outside the nucleus. For example, the presence of nuclear calcium is required for the induction of activity-dependent, CREB-mediated gene transcription (Hardingham et al. 1997; Chawla et al. 1998; Kornhauser et al. 2002). Additionally, activation and nuclear translocation of other regulators of transcription can be directly or indirectly induced by cytoplasmic calcium (Shibasaki et al. 1996; Meffert et al. 2003). The calcium-mediated activation of protein kinase cascades in the cytoplasm also activates CaMKs, PKA, MAPKs, and their subsequent translocation into the nucleus (Cohen and Greenberg 2008; Wayman et al. 2008; Xing et al. 1996; Impey et al. 1998).

As we can see, both nuclear calcium signaling and intermediate kinase activation are required to control the transcription of CREB, which is possibly the best-described transcription factor in neurons and plays a central role in activity-dependent synaptic plasticity. The convergence of multiple intracellular signaling cascades in neurons leads to the CREB activation, suggesting that this transcription factor is crucial for the integration of different inputs. CREB modulates the transcriptional response of the neuron, depending on the type and strength of the input and the current state of the neuron (Barco et al. 2007). The transcriptional regulation mediated by CREB has been implicated in persistent forms of synaptic plasticity like synaptic growth, as shown in *Aplysia* (Bailey and Kandel 1993). In this animal model, the addition of phosphorylated CREB-1 to cultured sensory neurons induces long-lasting synaptic rearrangements and the formation of new and stable synaptic contacts (Casadio et al. 1999).

Induction of LTP is associated with the generation of new synapses and with the enlargement of existing dendritic spines (Matsuzaki et al. 2004; Nägerl et al. 2004); this is very clear in brain slices from neonatal animals (Zhou et al. 2004), but it can also be observed in the adult brain in a more subtle way (Lang et al. 2004). However, when CREB-dependent gene expression is coupled to subthreshold synaptic activation, silent synapses are converted into active ones (Marie et al. 2005). In general, the gene expression cascade triggered by CREB provides molecular signals that orchestrate the stabilization and strengthening of synaptic connections (Lang et al. 2004). This includes the formation of new synapses and the remodeling of preexisting ones, both of which play an important role in long-term memory formation (Lang et al. 2004).

6 Participating Genes

It is well accepted that long-term memory formation requires transcription of specific genes into mRNAs and their translation into proteins. Some of these genes are the so-called immediate early genes (IEGs) that are rapidly, but transiently,

transcribed in response to a wide variety of cellular stimuli. IEGs are functionally defined as those genes that can be transcribed in the presence of protein synthesis inhibitors. Several IEGs have been identified, and they are of two types: the transcription factors, which regulate the transcription of other genes, such as c-fos, c-jun, zif268, and Egr-3; and the so-called "effector" IEGs, which act directly upon the cell to promote plastic changes, such as Arc, Narp, Homer, Cox-2, and Rheb (Lanahan and Worley 1998).

The transcription factor IEGs c-fos, c-jun, and zif/268 have been considered good candidates for the initial steps of learning-induced, persistent forms of synaptic plasticity (Abraham et al. 1991), because they are believed to trigger cascades of activity-dependent neuronal gene expression that can lead to plastic events in neurons. These genes are considered critical for memory consolidation (Abraham et al. 1991). Importantly, the patterns of activity that induce LTP are the same as those that induce some, but not all, of the IEGs (Worley et al. 1993). For example, zif268 expression in the hippocampus is triggered by the same pattern of activity that induces LTP (Worley et al. 1993), indicating that the thresholds for synaptic activation to induce the expression of specific transcription regulator genes in particular regions of the brain can be closely linked to synaptic plasticity. IEGs like c-fos are strongly induced in the hippocampus by experimental treatments used to produce models of epilepsy (Dragunow et al. 1992), and these manipulations are known to produce structural changes, such as mossy fiber sprouting in the hippocampus (Epsztein et al. 2005). It has also been found that the expression of c-fos precedes the morphological changes that occur in the cortex and are associated with motor skill learning. Consistent with this, in a mouse with a c-fos null mutation, mossy fiber sprouting cannot be induced as a result of kindling (Watanabe et al. 1996). These data suggest that c-fos might be able to trigger the expression of other genes related to structural synaptic plasticity, and it has been demonstrated that one such gene is BDNF (Dong et al. 2006).

Particularly interesting for the persistent forms of synaptic plasticity are the effector IEGs like *Arc*, *Homer,* and *Narp*. They all have been implicated in activity-dependent synaptic plasticity; however, the IEG Arc is of particular interest due to its exquisitely regulated, activity-dependent expression, and pivotal role in synaptic plasticity. Arc stands for activity-regulated, cytoskeleton-associated protein, but is also known as *Arg3.1*. It was originally identified in the hippocampus and cortex (Lyford et al. 1995; Link et al. 1995). Is an effector IEG, which means that Arc protein found in the cytoplasm exerts, by itself, a direct cellular function; unlike other IEGs, it is not a transcription factor. Transcription of Arc is induced after strong cellular activity and also by BDNF and NGF. It requires NMDA receptor activation and is regulated by acetylcholine (Lyford et al. 1995).

Another noteworthy feature of *Arc* is that its mRNA travels very rapidly to the dendritic tree (Wallace et al. 1998; Steward et al. 1998). In addition, *Arc* mRNA trafficking along the dendrites is seen selectively in the activated regions of the dendrite (Steward et al. 1998), and becomes enriched at the site of local synaptic activity, suggesting that Arc protein is locally synthesized. The activity-dependent induction of Arc mRNA and protein was an early clue that it had a role in memory

consolidation, so it is not surprising that the first studies on Arc concentrated on its regulation and function in the hippocampus. Arc also is involved in maintaining synaptic plasticity. Administration into the hippocampus of antisense oligonucleotides against *Arc* mRNA impairs LTP maintenance and consequently memory consolidation (Guzowski et al. 2000), and it is known that BDNF-induced LTP depends on the translation of Arc protein (Bramham and Messaoudi 2005). The *Arc* knock-out appears to confirm these observations, given that these animals show normal short-term memory and impaired long-term memory (Plath et al. 2001, 2006). Interestingly, *Arc* knock-outs also present impaired LTD maintenance, suggesting that Arc protein is important for stabilizing depressive changes in the efficacy of synapses. The infusion of Arc antisense oligodeoxynucleotides (ODNs) in the rat hippocampus blocked consolidation in a spatial memory task (Guzowski et al. 2000). Similarly, Arc antisense ODN infusion in the lateral amygdala blocked the consolidation of Pavlovian fear conditioning (Ploski et al. 2008). These findings suggest that Arc has a conserved role in information storage in limbic forebrain memory systems and is important for long-lasting synaptic plasticity and memory formation (Shepherd and Bear 2011).

Another interesting role of Arc in synaptic plasticity is related to its interaction with dynamin and endophilin, which are cytoskeletal proteins that regulate the endocytosis of AMPA receptors. By directly interacting with these proteins, Arc regulates the surface density of AMPA-type glutamate receptors (Chowdhury et al. 2006). One study shows that Arc overexpression in hippocampal cultures and slices results in downregulation and accelerated endocytosis of surface AMPARs (Shepherd and Bear 2011). In contrast, *Arc*−/− neurons have slower receptor endocytosis and abnormally high basal levels of surface GluR1. These findings support the view that activity-dependent Arc induction is involved in synaptic scaling. This is a homeostatic type of synaptic plasticity, and it refers to the erasure of nonspecific synaptic changes that occurred in nonstimulated synapses along the dendritic tree during the induction of activity-dependent synaptic change. The nonspecific changes are eliminated in order to maintain the specificity of the activity-dependent synaptic change (Shepherd and Bear 2011).

A very important feature of Arc is that it interacts with CaMKII and this interaction may be of great relevance for persistent forms of synaptic plasticity. When we studied the cell types that were able to express Arc, we found that all the cells that expressed Arc were also CaMKII-positive in regions such as the hippocampus and the cortex (Vazdarjanova et al. 2006). This indicated a possible interaction between these two plasticity-related proteins, Arc and CaMKII. Results obtained using fluorescence resonance energy transfer in cultured neurons have shown that the Arc and CaMKIIβ proteins can indeed interact but only in the dendritic spines (Okuno et al. 2012). Interestingly this interaction between Arc and CaMKII-β occurs in the nonactivated synapses. The presence of Arc in these nonactivated spines regulates both AMPA endocytosis and the activity of CaMKII, promoting synaptic scaling of the nonactivated spines, while in the activated spines CaMKII may promote structural synaptic changes (Okuno et al. 2012). This is known as "inverse synaptic tagging" a mechanism underlying a persistent form

of synaptic plasticity, known as synaptic scaling. This mechanism operates as a specific sensor for the inactive synapse-specific control of AMPA-R clearance at weaker synapses in plastic neurons, based on a local history of both activity and inactivity (Okuno et al. 2012). This is very significant evidence that contributes to a clear understanding of the mechanisms underlying synaptic tagging and the role of Arc in the stabilization of synaptic plasticity.

Regarding the possible role of Arc in remodeling the actin cytoskeleton of the spine, of particular interest is that Arc protein has high homology to alfa-spectrin, and it copurifies with F-actin (Steward and Worley 2001; Husi et al. 2000), both of which are important cytoskeletal proteins, which initially suggests a role in structural synaptic plasticity. In fact, Arc has clearly been implicated in remodeling the actin cytoskeleton in dendritic spines (Shepherd and Bear 2011). Thus, we can postulate that Arc expression may simultaneously reduce synaptic efficacy through AMPA receptor endocytosis, while increasing structural plasticity by favoring spine-type morphogenesis (Peebles et al. 2010).

Evidence has shown that LTP in the dentate gyrus is associated with a long-lasting increase in spine F-actin content, an increase in synapse diameter, and enhanced cofilin phosphorylation (Fukazawa et al. 2003), which promotes actin polymerization. Reversal of LTP after infusion of Arc antisense 2 h after high-frequency stimulation correlated with dephosphorylation of hyperphosphorylated cofilin and a loss of F-actin; however, Arc antisense infusion did not reverse LTP when the F-actin-stabilizing drug, jasplakinolide was present (Messaoudi et al. 2007).

Possible interactions of Arc with other actin-regulating proteins were investigated, and it was found that in neurons, Arc binds directly to Wave3, an actin-nucleating factor (Peebles 2009). Furthermore, it was demonstrated that reducing the expression of Wave3 leads to a decrease in dendritic length, a reduction in spine density, and an increase in the number of filopodia. The expression of Arc partially reversed these morphological effects supporting the idea that the opposing actions of Arc and Wave 3 interact to regulate synapse morphology. Together, these results suggest that Arc synthesis promotes F-actin polymerization and spine remodeling, although the details of the mechanism are still unknown.

As we previously noted, the transcription factor CREB regulates a variety of genes whose protein products control synaptic function, but the actual targets of CREB that participate in synaptic plasticity have not yet been fully identified. However, it has been recently demonstrated that expression of BDNF, a trophic factor known to be important for synaptic plasticity, is mediated by a CREB-family transcription factor-dependent mechanism (Tao et al. 1998). The neurotrophin family is a group of regulatory factors involved in cell development, survival, and repair, but one of the most interesting features of these proteins is their role in neural plasticity. The neurotrophin family includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophins 3 and 4 (NT3, NT4), and two other members were found in fish, NT6 and NT7 (McAllister 2002). Each neurotrophin has been shown to promote neurite outgrowth by responsive neurons in either in vitro or in vivo studies (Frade et al. 1999). BDNF is known to regulate the development of connections (Horch 2004) and the complexity and size of dendrites (McAllister et al. 1995) in the

cerebral cortex. The involvement of BDNF in synaptic plasticity has long been suspected, and it is known to regulate glutamatergic activity by increasing NMDA receptor phosphorylation (Slack et al. 2004). During development BDNF regulates neuronal proliferation, neuronal migration, axon path finding, dendritic growth, synapse formation, and maintenance of the synaptic contact (Thomas and Davies 2005). Interestingly, BDNF can be another central player in learning-induced structural plasticity, particularly since it is involved in spatial memory formation (Mizuno et al. 2000). Animals subjected to Morris Water maze training showed increased expression in the hippocampus of the mRNAs for BDNF (Mizuno et al. 2000; Kesslak et al. 1998) and also for its receptor TrkB (Gomez-Pinilla et al. 2001); this expression was observed only after a few days of training, but not before (Kesslak et al. 1998). This interesting result resembles our previous observations (Ramirez-Amaya et al. 1999, 2001) that when animals were overtrained for 4–5 days in the Morris water maze task, the number of mossy fiber buttons in the stratum oriens of the CA3 hippocampal region increased (Ramirez-Amaya et al. 2001). The striking similarity between BDNF expression after water maze training and our observation regarding mossy fiber sprouting suggests that BDNF is a component of the molecular mechanism that underlies spatial learning-induced structural plasticity in the hippocampus. Additional evidence for this is the demonstration that blockade of BDNF mRNA or its protein product (Mu et al. 1999) produced spatial memory impairments (Mizuno et al. 2000).

The properties of BDNF make it a prominent regulator of persistent forms of synaptic plasticity. However, BDNF does not act alone, but instead interacts with other molecules that we have reviewed above. For example, it has been observed that BDNF induces the synthesis of Arc in synaptoneurosomes (Yin et al. 2002); also, BDNF induction of LTP depends on Arc protein translation (Bramham and Messaoudi 2005), suggesting an important role of Arc in BDNF-induced synaptic plasticity. An interaction between BDNF and CaMKII has also been observed. In cultured neurons using a GFP reporter of CaMKII, BDNF induces the translation of CaMKII in dendritic spines (Aakalu et al. 2001). With this data in hand, Braham and Messaudi (Bramham and Messaoudi 2005) suggested that CaMKII, Arc, and BDNF may interact in dendrites to mediate long-term synaptic plasticity. They have even proposed that Arc should persist in some way in order to consolidate its plastic effect on dendrites. Here, we have presented evidence indicating that Arc may perform this function in two different ways: first, by the offline reactivation of Arc expression in an apparently progressively smaller ensemble of neurons, and second, by the sustained transcription of Arc that apparently occurs only in the hippocampal dentate gyrus. Both mechanisms may interact with CaMKII, Ca^{2+} influx, and other events triggered by NMDA receptor activation.

7 Conclusion

After stimulation, the activity evoked in the neural network may become reverberatory, and this will lead to activity-dependent synaptic plasticity of the participating units that will form a "neural ensemble". According to the current theory, this is the main mechanism through which the neural network processes and stores information, resulting in what we call memory. Synaptic plasticity is a mechanism that is enabled in the physiological neural network; prominent examples are LTP and LTD, but more persistent types of plasticity such as homeostatic plasticity and structural synaptic changes may underlie long-term memory. These persistent types of synaptic plasticity may require particular behavioral and environmental conditions. Structural synaptic changes observed after robust behavioral experience may be related to long-term memory, but also possibly to functional improvement of the neural network.

The concept of a population code is supported by measurements of multiple, single-unit recordings that can be analyzed in a way that allows us to predict the behavioral output of the animal by simply analyzing the electrophysiological signal, and the bigger the size of the ensemble, the more reliable the prediction. Moreover, when detecting the activity of neurons using IEG staining methods, it is observed that the neuronal populations that are recruited for information processing leading to memory storage, are the same as those later recruited by the same or similar stimuli. This indicates clearly that neuronal populations are the ones that encode and store information.

The notion of sparse coding implies that for efficient memory storage and retrieval, the neural network must use a small group of neurons that will ultimately undergo synaptic changes, suggesting that the cellular mechanisms that lead to persistent forms of synaptic plasticity may occur in a subset of the originally activated cells, because the initial recruitment is too large, particularly in regions such as the neocortex and CA regions in the hippocampus; the networks in these areas may select the plastic neurons through the synergic action of different signal transduction pathways working together during offline reactivation periods.

In other regions such as the hippocampal DG, the network initially recruits a sparse number of neurons, but those selected are more likely to achieve synaptic plasticity, since the transcription of some genes, such as Arc, is maintained for a prolonged period of time. It is not yet clear how many neurons ultimately undergo synaptic plasticity and become responsible for reproducing the acquired patterns of activity after long intervals, but it is possible that the system uses a sparse code for long-term memory to achieve efficient storage and retrieval. Accurate methods to count these units are required.

The different temporal fates of memory depend on the persistence of the synaptic changes. This idea assumes that short- and long-term memory share similar principles, but the mechanisms that lead to either transitory or persistent synaptic changes may differ. In both network systems, however, the persistent forms of synaptic plasticity might be driven by the synergic action of multiple signal transduction

pathways that, in some networks cause molecular activity waves and in the others, sustain the activity of particular factors. Synaptic inputs that result from the reverberatory activity of the network trigger one or more of these neural transduction pathways that include NMDA, AMPA receptors that lack GluR2, metabotropic glutamate receptors, acetylcholine, and dopamine. These triggers synergistically give rise to and modulate different cellular events that culminate in a persistent form of synaptic plasticity (Fig. 1).

Presynaptic glutamate release followed by depolarization of the postsynaptic neuron produces Ca^{2+} influx through voltage-dependent Ca^{2+} channels and NMDARs. Ca^{2+} signals are complex and fast, and they need to be translated into graded and steady signals, such as the one provided by CaMKII. This remarkable Ca^{2+} signal transducer is a kinase that, through phosphorylation, regulates several proteins; among them, CaMKII directly regulates the stability of actin and other actin polymerization and depolymerization factors such as cofilin and profilin, inducing structural remodeling of the synapse. CaMKII also phosphorylates the AMPAR GluR1 subunit, increasing the number of functional AMPARs. Principally, CaMKII plays a central role in orchestrating the signals leading to synaptic plasticity, because it also regulates other signal transduction pathways, including the one associated with members of the Rho family of small GTPases and known to be involved in transcriptional regulation.

Other triggering signals leading to this pathway are dopamine D1 receptors (D1Rs), muscarinic acetylcholine receptors (M2R and M4R), and β-adrenergic receptors (βRs). These receptors induce adenylate cyclase activity. The cyclic AMP produced by adenylyl cyclase activates protein kinase A (PKA), which subsequently phosphorylates Rap GTPases. On the other hand, muscarinic acetylcholine receptors M1 and M3 are coupled to the Gq protein, which activates phospholipase C (PLC) and promotes the formation of IP3 and DAG. IP3 triggers Ca^{2+} release from intracellular stores, and both Ca^{2+} and DAG activate protein kinase C. Once activated, PKC can activate Raf kinases, which are also members of the MAPK pathway.

We can see here that all these signal transduction pathways converge in the small GTPase pathway that includes Rap-1 or Ras which, in turn, induces Raf phosphorylation. The Raf isoforms then phosphorylate MEK1/2, which subsequently phosphorylates the mitogen-activated protein kinase 2–3, also known as ERK1/2. Phosphorylated ERK1 and ERK2 are released from MEK1/2 and either dimerize with a second ERK molecule or remain monomeric. Interestingly, in the cytoplasm this pathway also modulates protein translation and protein trafficking to the membrane. Also both forms of ERK1 and ERK2 can probably enter the nucleus, where they are important regulators of gene transcription. Another, indirect nuclear ERK1/2-target is CREB, which is phosphorylated by the ERK1/2 substrates RSK2 and MSK1. Phosphorylation of CREB at Ser133 promotes the recruitment of the coactivator CBP and initiates the transcription of target genes.

At the transcriptional level, the molecular events are no less complex. There are several activity-regulated genes that encode other transcription factors which, in turn, promote the transcription of plasticity-related genes, such as BDNF,

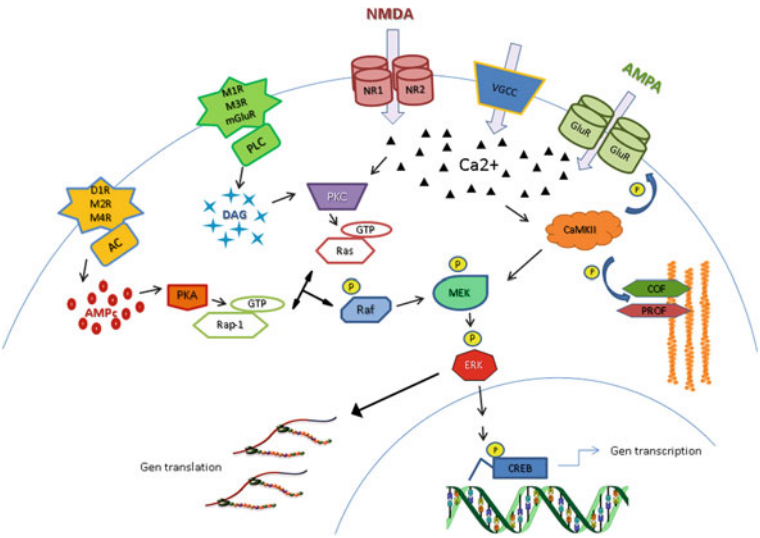

Fig. 1 Molecular mechanisms underlying structural synaptic plasticity: several signal transduction pathways that include a variety of transmembrane receptors are activated during information processing in the neural network. One pathway involves NMDA and AMPA receptors, and with the participation of voltage-dependent calcium channels, it triggers rapid Ca^{2+} influx; this in turn activates CaMKII, which is involved in remodeling spine structure since it interacts with the actin cytoskeleton and phosphorylates actin polymerization regulator proteins such as cofilin or profilin. This pathway also activates MEK, which is part of the MAP kinase pathways. In addition, the activation of muscarinic (M2 and M4) and dopaminergic receptors is associated with the activation of adenylate cyclase (AC) resulting in increased levels of cAMP; this second messenger activates PKA which, in turn, phosphorylates GTPases such as Rap-1. On the other hand, metabotropic receptors such as cholinergic M1 and M3 and metabotropic glutamate receptors (mGluR) are associated with phospholipase C (PLC), and their activation induces the production of the second messengers IP3 and DAG. This latter activates PKC, which phosphorylates certain GTPases such as Ras. It is clear that these pathways, involved in structural synaptic plasticity, converge in the activation of the MAP kinase pathway. The GTPase family (Rap-1, Ras, etc.) can phosphorylate Raf, which in turn phosphorylates MEK. Here at MEK is where the glutamate receptor activation, both ionotropic and metabotropic, converge. MEK phosphorylates ERK 1/2 triggering its downstream effects, such as translation regulation in the cytoplasm and gene transcription in the nucleus. The regulation of both translation and gene transcription may modulate important proteins related to structural synaptic plasticity. A prominent example is the transcription factor CREB, which regulates many other genes important for synaptic plasticity. Another way ERK 1/2 regulates transcription is through epigenetic transcription regulation, by phosphorylating H3. Various genes such as the IEG Arc, Zif268, and BDNF are involved in the molecular mechanisms underlying structural synaptic plasticity. Finally, cofilin (cof) and profilin (prof) are associated with the polymerization and depolymerization of actin filaments and their regulation is important for structural synaptic plasticity

glutamate receptors, and many other activity-regulated genes including Arc and Homer 1a which encode effector proteins that have specific roles in dendritic function or coordinate activity-dependent synaptic plasticity. Because this gene expression program is the molecular basis of the mechanisms underlying learning

and memory, the signaling pathways that link the triggers, e.g., calcium influx, with the regulation of transcription, need to be extensively investigated in order to accomplish a detailed characterization of the cellular and molecular events that lead to long-term memory. Exquisitely detailed molecular modeling will be required to understand how these complex events are orchestrated and ultimately, to identify the molecular principles that shape the biological neural network.

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Neurotrophins and Synaptic Plasticity

Andrea Gómez-Palacio-Schjetnan and Martha L. Escobar

Abstract It has been suggested that long-term modifications of synaptic transmission constitute the foundation of the processes by which information is stored in the central nervous system. A group of proteins called neurotrophins are considered powerful molecular mediators in central synaptic plasticity. Among these, brain-derived neurotrophic factor (BDNF) as well as neurotrophin-3 (NT-3) have emerged as having key roles in the neurobiological mechanisms related to learning and memory. In this chapter, we review the studies that have represented a significant step forward in understanding the role played by BDNF and NT-3 in long-term synaptic plasticity. The effects of BDNF and NT-3 on synaptic plasticity can be of a permissive nature, establishing the conditions under which plastic changes can take place, or it may be instructive, directly modifying the communication and morphology of synapses. The actions carried out by BDNF include its capacity to contribute to the stabilization and maturation of already-existing synapses, as well as to generate new synaptic contacts. One important finding that highlights the participation of these neurotrophins in synaptic plasticity is the observation that adding BDNF or NT-3 gives rise to drastic long-term increases in synaptic transmission, similar to the long-term potentiation in the hippocampus and neocortex of mammals. Because neurotrophins modulate both the electrical properties and the structural organization of the synapse, these proteins have been considered important biological markers of learning and memory processes.

Keywords Neurotrophins · Synaptic plasticity · Homeostatic plasticity · BDNF · NT-3

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Curr Topics Behav Neurosci (2013) 15: 117–136
DOI: 10.1007/7854_2012_231

Published Online: 22 March 2013

Contents

1 Introduction

Over the last few years, one of the most challenging subjects in the field of neuroscience has been to describe how the central nervous system (CNS) stores and processes information for long periods. Nowadays, a consensus has been reached, placing the long-term structural and functional synaptic modifications as the primary mechanisms underlying the process of long-term storage of information (Escobar and Derrick 2007). Such modifications have special relevance in the study of learning and memory processes due to their place as the prevailing mechanisms of plasticity. Recently, a group of proteins called neurotrophins have emerged as potent molecular mediators of behavioral modifications related to environmental interactions. In this chapter, we compiled some of the most relevant studies in the field that place neurotrophins as having a crucial role in long-term synaptic plasticity (Lu et al. 2005).

2 Neurotrophins and Their Receptors: General Description

Cellular diversity is derived from the orchestration of complex processes such as cellular proliferation, differentiation, growth, migration, and synaptic formation. Among all the chemical messengers involved in such processes, a group of proteins has caught researchers' attention due to their crucial involvement eliciting the survival, cell growth, and maintenance of the functional capacities of specific neuronal populations. This family of proteins, known as neurotrophins, contains nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), as well as the recently identified neurotrophin-6 (NT-6) and neurotrophin-7 (NT-7). The characterization of NGF allowed the subsequent identification of the other members of this family.

Neurotrophins have a long tri-dimensional structure with three disulfide bonds, one of which forms a characteristic connecting cystine knot (Gotz et al. 1994; Ibanez 1994; Nilsson et al. 1998).

Initially, neurotrophins are synthesized as precursor proteins (proneurotrophins) in a similar fashion to other neuropeptides. They are then processed by a diverse number of enzymes which convert the proneurotrophins into mature neurotrophins once they are released to the extracellular space (Lessmann et al. 2003). In their mature form, each of these proteins forms a complex with a twin molecule (each with an approximate molecular weight of 13 kDa), forming a dimeric structure that allows the activation of specific receptors (Chao 2003). Neurotrophins act through two types of receptors: tyrosine kinase receptors, with a high affinity for mature neurotrophins, and p75 receptors, with a low affinity for mature neurotrophins and a high affinity for immature or precursor neurotrophins (Lu et al. 2005). It has also been observed that the p75 receptor can form a heteromeric complex with the Trk receptors, generating modifications of their affinity constants to the specific neurotrophins (Chao 2003).

Recent studies have suggested that proneurotrophins, by interacting with the p75 receptors, exert opposite biological effects as compared to mature proteins. This implies that the proteolytic cleavage of the proneurotrophins represents a mechanism of control directing neurotrophin actions (Lu et al. 2005). One or more members of this group of proteins can activate the various types of Trk receptors. Accordingly, TrkA is mainly activated by NGF, TrkB can be activated by BDNF and NT-4/5, and TrkC is activated by NT-3 (see Fig. 1). An interesting characteristic of neurotrophin actions is their capacity to autoregulate their production as well as to regulate the production of other members of this group of proteins (Canossa et al. 1997; Patz and Wahle 2004). A study published by Patz and Wahle (2004) exemplifies this regulation. They report that the BDNF application enhances BDNF, NGF, and NT-3 expression in cortical neurons (Patz and Wahle 2004).

In addition to their important role in early nervous system development, in recent years, neurotrophins have been demonstrated to be extensively involved in long-term synaptic plasticity in the adult nervous system (Bramham et al. 1996; Schinder and Poo 2000).

3 Role of BDNF and NT-3 in Synaptic Efficacy

Evidence regarding the role of BDNF in neuronal plasticity suggests that this neurotrophin contributes to long-term plasticity in both a permissive and instructive manner. It does so by establishing the conditions that allow plastic changes, as well as by exerting direct effects that bring about changes in the communication and morphology of the synapses (Schinder and Poo 2000; Poo 2001; Soule et al. 2006).

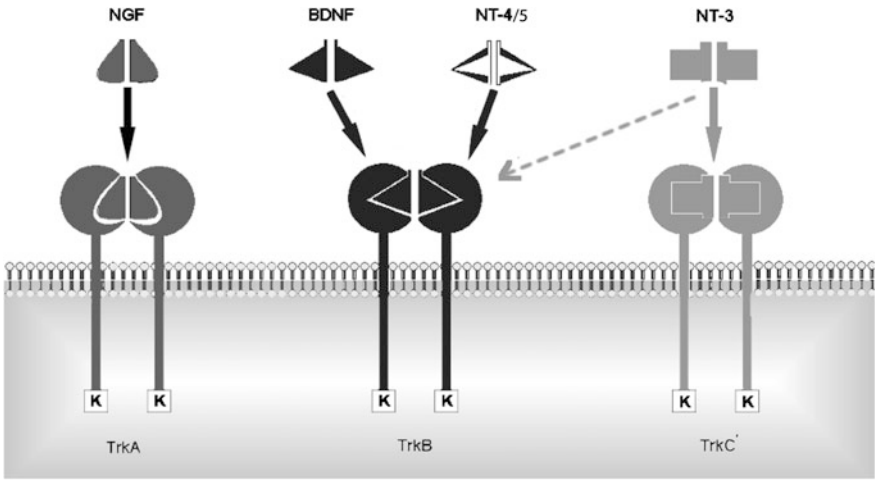

Fig. 1 Cartoon showing Trk receptors that are activated by different members of the neurotrophin family. The image is a representation of the interaction of neurotrophins (nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT-3), and neurotrophin 4/5 (NT-4/5)) with its high affinity receptor. Each of these proteins forms a dimeric structure that allows activation of specific receptors. Trk receptors are activated preferably by one or more members of this family of proteins. Thus, the TrkA receptor interacts mainly with NGF, TrkB mainly with BDNF and NT-4/5, and TrkC with NT-3. [K] represents the intracellular portion of tyrosine kinase. *Arrows* represent main interactions of the neurotrophins with Trk receptors. *Dotted arrows* represent alternative interactions between the specific neurotrophins and Trk receptors

In recent years, studies have shown that BDNF expression and secretion requires repetitive stimulation patterns to promote increments of intracellular calcium in a similar fashion to the patterns required for the secretion and the release of other neuropeptides (such as vasopressin and opioid peptides) (Lessmann et al. 2003). In experimental models of epilepsy, it has been reported that increases in the expression of both BDNF mRNA and protein occur after induction of an epileptiform crisis (Morimoto et al. 1998; Binder et al. 2001; Danzer and McNamara 2004). These increases are directly proportional to the animal status epilepticus in the kindling model and are related to increases in intracellular calcium levels (Mhyre and Applegate 2003; Poulsen et al. 2004).

Recent evidence suggests that BDNF is a protein critically involved in regulating synaptic plasticity-related mechanisms (Pang et al. 2004; Lu et al. 2005). This is supported by findings that the induction of long-term potentiation (LTP) results in increased levels of BDNF and its TrkB receptor mRNA's (Bramham et al. 1996; Schinder and Poo 2000; Gooney and Lynch 2001; Poo 2001; Lessmann et al. 2003; Nagappan and Lu 2005). This was the case in a study by Bramham et al. (1996), which observed that high-frequency stimulation delivered to the perforant pathway increased BDNF and TrkB mRNA's at the dentate gyrus and hippocampal CA3 area, in vivo. Similar observations have been reported by Lee

and colleagues after inducing associative LTP (Lee et al. 2005). In addition, it has been shown that mouse strains lacking the gene that encodes BDNF exhibit a significant decrease in the induction of hippocampal LTP (Korte et al. 1995; Patterson et al. 1996; Monteggia et al. 2004). These deficiencies were corrected by the exogenous administration of BDNF (Patterson et al. 1996; Pang et al. 2004). Recently, Monteggia et al. (2004) reported a decrease in the expression and maintenance of LTP in adult animals, in which a selective blockade of BDNF expression was induced.

Concerning the patterns of secretion of BDNF, research suggests that this neurotrophin may be secreted both in a constitutive and regulated manner (Poo 2001; Lessmann et al. 2003). The regulated secretion of this neurotrophin is strongly related to LTP, with a 98 % increase in secretion having been observed in cultured hippocampal neurons when LTP was induced (Brigadski et al. 2005). Secretion triggered by the induction of LTP is also closely correlated with the stimulation pattern applied. That is, high-frequency activity capable of inducing late phase LTP (L-LTP, with duration ranging from several hours to months) requires prolonged secretion of BDNF (between 5–12 min). Stimulation that generates early LTP (E-LTP, which lasts several minutes to 2 or 3 h) requires a less pronounced secretion of BDNF (about 1 min) (Hartmann et al. 2001; Aicardi et al. 2004). The secretion of this neurotrophin is also highly dependent on intracellular calcium concentration (Pozzo-Miller 2006), which in turn is regulated through ionotropic glutamate receptors and/or the release of intracellular calcium stores (Bramham et al. 1996; Hartmann et al. 2001; Gartner and Staiger 2002; Lessmann et al. 2003; Thomas and Davies 2005). It is also important to note that the activity-regulated secretion is observed both in the postsynapses and the presynapses, highlighting the paracrine and autocrine actions of BDNF (Gooney et al. 2002; Lessmann et al. 2003).

The effects of BDNF on synaptic plasticity are inevitably related to the activation of its receptor TrkB (Chao 2003). In this regard, it has been reported that an increase in transcription, translation, and transportation of TrkB after high-frequency activity is capable of inducing LTP. The insertion and endocytosis of this receptor (necessary for the activation of the tyrosine residues) is also regulated by synaptic activity. Moreover, the secretion of BDNF induces lateral movement of the receptors to the sites where the plastic changes are occurring (Nagappan and Lu 2005).

Another important finding concerning the involvement of BDNF in synaptic plasticity is the observation that its administration produces dramatic long-term increases in synaptic transmission (LTP-like) in both in vivo and in vitro preparations of hippocampal neurons of rodents (Kang and Schuman 1995; Messaoudi et al. 1998; Ying et al. 2002; Bramham and Messaoudi 2005; Gomez-Palacio-Schjetnan and Escobar 2008). As mentioned earlier, BDNF and its high affinity receptor TrkB are expressed abundantly in neurons in the neocortex of mammals (Yan et al. 1997). In this respect, it has been observed that BDNF induces prolonged increases of synaptic efficacy in the visual cortex (Jiang et al. 2001) and recently in our laboratory, in the insular cortex, linking these effects with TrkB

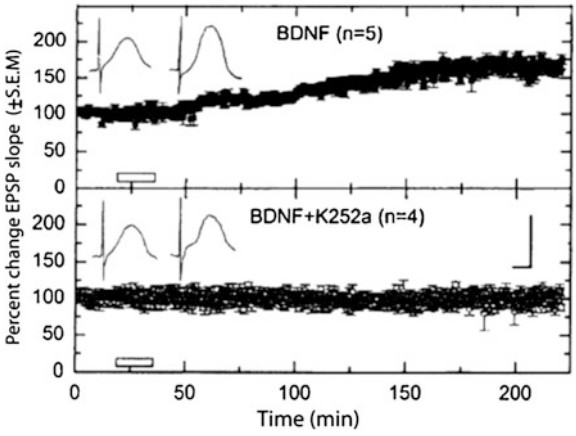

Fig. 2 Effects of acute brain-derived neurotrophic factor (BDNF) infusion on amygdalo-cortical (Bla-IC) responses in vivo. Plot of insular cortex evoked responses (measured by EPSP) from animals that received BDNF (3 μg/3 μl) (BDNF) and BDNF + K252a (3 μg/3 μl) (BDNF + K252a). After BDNF infusion, EPSP slope values were significantly elevated above baseline at 15 min and climbed gradually to a stable plateau at 2 h. No changes were observed in the BDNF + K252a group, reflecting the role of TrkB receptor on BDNF actions. The bars indicate the period of microinfusion (15 min). Scale: 9 ms, 0.5 mV. Note that K252a is an inhibitor of the Trk receptors. Modified from Escobar et al. (2003)

receptor activation through the infusion of Trk receptor inhibitors (Escobar et al. 2003) (see Fig. 2). BDNF is also involved in the regulation of cortical excitability (Rutherford et al. 1998) and elevates the magnitude of tetanic-induced LTP in the visual cortex (Akaneya et al. 1997; Huber et al. 1998).

Although rapid effects in synaptic transmission elicited by BDNF through activation of voltage-dependent sodium channels have been reported (Rose et al. 2004), numerous experiments indicate that BDNF is a key factor in the late phase of LTP (Schinder and Poo 2000; Lu et al. 2005; Soule et al. 2006). Pang et al. (2004) observed that administration of BDNF was able to restore the late phase of hippocampal LTP, previously depressed by the administration of protein synthesis inhibitors. Similarly, Barco et al. (2005) reported the restoration of L-LTP in animals lacking the gene encoding the transcription factor CREB (cAMP response element binding) after administration of BDNF (Barco et al. 2005). These studies suggest that BDNF is a key component of protein synthesis underlying L-LTP.

In recent years, the role of NT-3 in synaptic efficacy has gained considerable attention. It has been described that, at cultured neuromuscular synapses, acute exposure to NT-3 rapidly potentiates synaptic transmission (Lohof et al. 1993). Long-term treatment with NT-3 produced significant changes in synaptic efficacy. Such effects were dependent on interaction with the TrkC receptors (Wang et al. 1995; Lu 2004; Je et al. 2006, 2010). In the adult mammalian CNS, the hippocampus is a prominent site of expression of NT-3 and its receptor, TrkC (Maisonpierre et al. 1990a, b; Friedman et al. 1991; Sandell et al. 1994). It has also

been demonstrated that the expression of the NT-3 gene in the adult brain is highly confined to the dentate gyrus (DG) (Lauterborn et al. 1994; Zhang et al. 2007). Neuronal activity such as LTP-induction can modulate the expression of NT-3 and TrkC mRNA in the hippocampus (Patterson et al. 1992; Bramham et al. 1996), which suggests that this neurotrophin may play a significant role in adult synaptic plasticity. In addition, it has been reported that NT-3 infusion induces a robust, long-lasting enhancement of synaptic transmission at the Schaffer collateral pathway in vitro (Kang and Schuman 1995). However, direct evidence regarding the role of NT-3 in adult synaptic plasticity in vivo remains scarce. In this regard, a recent study conducted in our laboratory shows that NT-3 administration elicits a long-term enhancement of the MF-CA3 synaptic transmission in anesthetized adult rats (Ramos-Languren and Escobar submitted).

4 Role of BDNF and NT-3 on Brain Architecture

In 1896, Santiago Ramon y Cajal (quoted by De Felipe) presciently proposed that information is stored in our brain through changes in the strength of synaptic transmission-dependent activity, which may result in morphological modifications of the system (DeFelipe 2006). Today, changes in synaptic structure are recognized as an important expression of plasticity (Lamprecht and LeDoux 2004).

Over the last decade, many fascinating studies have shown that new neurons are actively produced in the subventricular zone (SVZ) of the lateral ventricle and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus of the adult mammalian brain (Gould et al. 1999). One of the most notable roles of neurotrophic factors is their participation in the mechanisms regulating this dramatic form of synaptic plasticity (Lee and Son 2009). Among these, BDNF has been extensively related to adult hippocampal neurogenesis in both, the proliferation phase as well as the survival and differentiation of newly generated cells. For example, infusion of BDNF has a neurogenic effect in both the SVZ and the dentate gyrus (Pinnock and Herbert 2008; Im et al. 2010). Coincidently, knockout of BDNF or TrkB in mice resulted in a 50 % reduction of newly generated neurons (Sairanen et al. 2005). Importantly, BDNF signaling has also been implicated in the maturation of new neurons, allowing the integration of such cells into functional hippocampal circuits (Bergami et al. 2008; Babu et al. 2009). These actions appear to be modulated by 5-HT expression (Bergami et al. 2008). The trophic regulation that NT-3 exerts in early developmental stages has led to the idea that this neurotrophin would also be implicated in adult neurogenesis. Although the evidence remains scarce, there are some studies that indicate the role of NT-3 in neuronal proliferation in the hippocampus (Shimazu et al. 2006) as well as differentiation of neural precursors in the spinal cord (Gu et al. 2009).

The most consistently observed effects of BDNF include the ability to contribute to the stabilization and maturation of existing synapses, as well as generating new synaptic contacts (Vicario-Abejon et al. 2002). Tolwani et al. (2002)

have reported dramatic increases in the arborization of hippocampal dendrites and axons in both young and adult animals coinciding with the presence of BDNF (Tolwani et al. 2002). Correspondingly, significant decreases in axonal arborization of hippocampal granule cells were observed in mice lacking the gene encoding TrkB (Otal et al. 2005). Using the visual system neurons of transgenic mice carrying synaptobrevin 2 conjugated with green fluorescent protein (GFP), it has been shown that BDNF aids in the arborization and maturation of neurites, as well as increasing the number of synaptic sites (Labelle and Leclerc 2000; Alsina et al. 2001). Furthermore, the presence of BDNF has been correlated with an increase in the number of axonal branches of hippocampal mossy fibers (Danzer and McNamara 2004), and in the number of dendritic spines in hippocampal pyramidal cells (Ji et al. 2005).

Although NT-3 has been consistently associated with the promotion of neurogenesis, neurite development and maintenance, as well as the establishment of synaptic connections (McAllister et al. 1999; Shimazu et al. 2006), the role of this neurotrophin in morphological modifications associated with adult synaptic plasticity needs further elucidation. Nevertheless, recently it has been reported that the lack of TrkC results in smaller and less complex mossy fiber buttons, as well as in a reduced number of synaptic vesicles, altering the synaptogenesis and maturation of mossy fiber terminals (Martinez et al. 1998; Otal et al. 2005). In a complementary observation, it was shown that a prolonged intraventricular infusion of NT-3 triggers mossy fiber sprouting in the inner molecular layer of the dentate gyrus and the stratum oriens of the CA3 region (Xu et al. 2002).

Research conducted by our team has recently implicated both BDNF and NT-3 in adult mossy fiber synaptogenesis, 7 days after neurotrophin-induced synaptic long-term enhancement (Ramos-Languren and Escobar submitted; Schjetnan and Escobar 2012; Gomez-Palacio-Schjetnan and Escobar 2008).

5 About the Mechanisms

It has been established that BDNF has a direct impact on synaptic efficacy, acting on both presynaptic and postsynaptic cells. For example, presynaptic BDNF increases neurotransmitter release (via phosphorylation of *release related proteins*) (Tyler et al. 2002b, 2006; Alder et al. 2005), while both BDNF and NT-3 have been involved in increasing glutamatergic currents in vitro (Paul et al. 2001). Their actions in the postsynaptic cell include glutamate receptor phosphorylation of both N-methyl-D-aspartate (NMDA) and non-NMDA type (Alder et al. 2005), retrograde messenger production, elevated calcium levels, and protein synthesis activation (Lessmann 1998; Wang et al. 2002; Yamada et al. 2002a) (see Fig. 3).

Although the signaling pathways activated by the interaction of BDNF and NT-3 with its receptors during learning and memory encoding are not fully understood, it has been reported that Trk activation triggers two different signaling cascades involved in plastic modifications. The first relates to the activation of the MAPK's

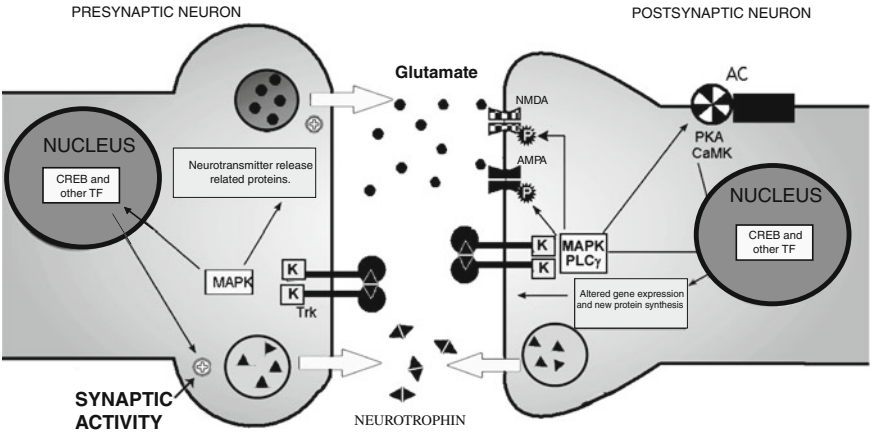

Fig. 3 Model of the pre- and postsynaptic actions of neurotrophins during synaptic plasticity. Neuronal activity increases the expression and release of the neurotrophins (in particular brain-derived neurotrophic factor; BDNF and neurotrophin 3; NT-3) in the pre- and postsynaptic sites. Neurotrophins bind to their Trk receptors, activating signaling cascades that include the mitogen-activated protein kinases (MAPK) and the phospholipase C γ (PLCγ) pathways. One of the main effects of BDNF and NT-3 in the presynapse is the facilitation of neurotransmitter release. Within the postsynaptic effects of these neurotrophins, the phosphorylation (P) of the N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA) is substantial. Pre- and postsynaptic actions of both neurotrophins in the long-term stimulate messenger RNA (mRNA) and proteins synthesis through the activation of transcription factors (TF). AC: adenylate cyclase, CREB: binding element responsive to cyclic AMP, FT: transcription factors

signaling pathway (Reichardt 2006). Activation of the TrkB results in the auto-phosphorylation of its SH2 intracellular tyrosine residue. This allows Grb2/SOS complex formation, which in turn activates a G protein ras that initiates the phosphorylation of MAPK proteins (such as MEK and ERK) through the activation of protein kinase Raf. This mechanism culminates in the activation of ribosomal S6 kinase (RSK), which phosphorylates CREB, thus upregulating transcription and the subsequent synthesis of new proteins (Finkbeiner et al. 1997) (see Fig. 4).

The second pathway triggered by activation of the TrkB receptor during modulation of synaptic plasticity concerns the activation of phospholipase Cγ (PLCγ) (Gartner et al. 2006). PLCγ cleaves phosphatidylinositol 4,5-bisphosphate (PIP-$_2$), which generates diacylglycerol (DAG) and inositol 1,4,5 triphosphate (IP3). IP3 induces the release of calcium from the intracellular pools, raising calcium levels in the cytosol and promoting the activation of calcium-dependent protein kinases. DAG activates protein kinase C, initiating the MAPK cascade which, as we mentioned in previous lines, phosphorylates CREB and upregulates protein production (Minichiello et al. 2002).

The mechanisms elicited by the NT-3/TrkC interaction have shown to regulate long-term synaptic potentiation by activation of PI-3 kinase (PI-3 K) and PLC-γ

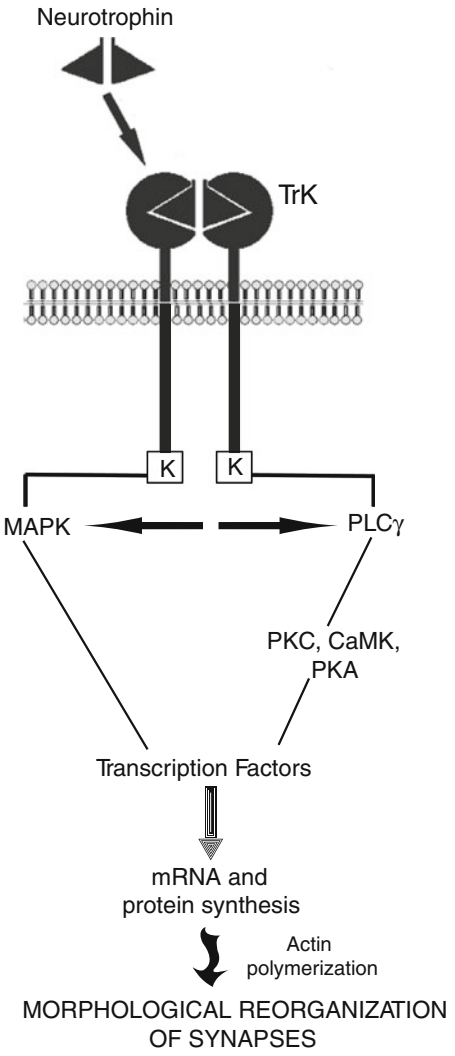

Fig. 4 Intracellular signaling pathways triggered by neurotrophins during synaptic plasticity. Trk receptors mediate synaptic plasticity, both through the mitogen-activated protein kinase (MAPK) pathway and activation of phospholipase C gamma (PLCγ). These mechanisms culminate in the activation of transcription factors and initiation and synthesis of new proteins, including members of the neurotrophin family. The activation of transcription factors results in the promotion of actin polymerization, crucial to induce structural modifications of the synapses. The signaling pathways activated by the Trk receptors often converge (*thin arrows*) during long-term synaptic plasticity. [K] represents the intracellular portion of tyrosine kinase. [MAPK] Mitogen-activated protein kinase. [PLC] phospholipase C gamma. [PKC] Protein kinase C (activated by calcium). [CaMK] Calcium/calmodulin-dependent protein kinase. [PKA] Protein kinase A (activated by cyclic AMP). [mRNA] messenger RNA

pathways, as well as mammalian target of rapamycin (mTOR)-dependent protein synthesis (Je et al. 2005). Additionally, Je et al. (2006) described how NT-3, through TrkC, modulates structural and functional plasticity via the Rap1-mitogen-activated protein kinase (Rap-1-MAPK) and Ca2 +/calmodulin-dependent kinase IV-cAMP response element binding protein (CaMKIV-CREB) pathways, respectively (see Fig. 4).

Proteins whose production is induced by activation of the CREB transcription factor are often involved in regulating morphological modifications and new synaptic connections. For example, the ability for neurons to undergo such morphological changes depends greatly on the availability and integrity of cytoskeletal

constituents, among which are the actin microfilaments (Lamprecht and LeDoux 2004). BDNF increases actin polymerization-related proteins (Labelle and Leclerc 2000; Avwenagha et al. 2003; Gehler et al. 2004). The action of the TrkB receptor regulates actin production and directly promotes its interaction with synapsin I (the protein found on the membrane of synaptic vesicles capable of interacting with actin filaments) (Perron and Bixby 1999; Jovanovic et al. 2000; Ohira et al. 2006). It has recently been reported that one of the long-term effects of BDNF, as part of its function in so-called synaptic consolidation, lies in the local synthesis of the Arc protein (activity-regulated protein associated with the cytoskeleton), which acts as a regulator during the formation of actin filaments (Soule et al. 2006).

The number of vesicles docked at the presynaptic membrane has been considered as a parameter of maturity during the formation of new synapses. As evidence of its role in the maturation of neurons, it has been observed that BDNF increases the number of vesicles coupled to the neuronal membrane (Collin et al. 2001; Tyler and Pozzo-Miller 2001; Tyler et al. 2006). As well, BDNF modulates the neurotransmitter release machinery regulating post-transcriptional stabilization of the SNARE complex (the protein complex that regulates the fusion of synaptic vesicles, allowing the release of neurotransmitter) (Tartaglia et al. 2001; Yamada et al. 2002b; Pozzo-Miller 2006; Ring et al. 2006). Phosphorylation of synapsins by protein MAPK (mitogen-activated protein kinases) is one of the main mechanisms by which BDNF stimulates the release of neurotransmitter (Jovanovic et al. 2000; Ploughman et al. 2005). BDNF has also been reported to have postsynaptic actions. This has been demonstrated in the case of changes in expression and insertion of the AMPA-type glutamate receptors (α-amino-3-hydroxy-5-methyl-4-isoxasol propionate) (Narisawa-Saito et al. 2002) and NMDA subunits (Lin et al. 1998; Haddad 2005).

6 Two Neurotrophins in Animal Behavior

Memory involves changes in the electrical properties of synapses, as well as structural changes. Given that BDNF modulates both expressions of synaptic plasticity, this neurotrophin has been considered an important marker of learning and memory processes (McAllister et al. 1999; Tyler et al. 2002a; Yamada et al. 2002b). In this regard, a series of studies to this effect have shown that spatial learning tasks resulted in increased levels of BDNF (Kesslak et al. 1998; Gobbo and O'Mara 2005) and its receptor TrkB mRNA's in the hippocampus (Gomez-Pinilla et al. 2001). Using in situ hybridization, a rapid and selective increase in BDNF expression has been observed in the hippocampal CA1 during contextual learning (Hall et al. 2000). Intraventricular infusion of BDNF antibodies were shown to cause significant decreases in the rodents performance on spatial learning tasks (Morris water maze (Mu et al. 1999). Similarly, in animals in which a blockade of the BDNF gene had been induced, there were considerable decrements in their performance on hippocampal-dependent memory tasks (Monteggia et al.

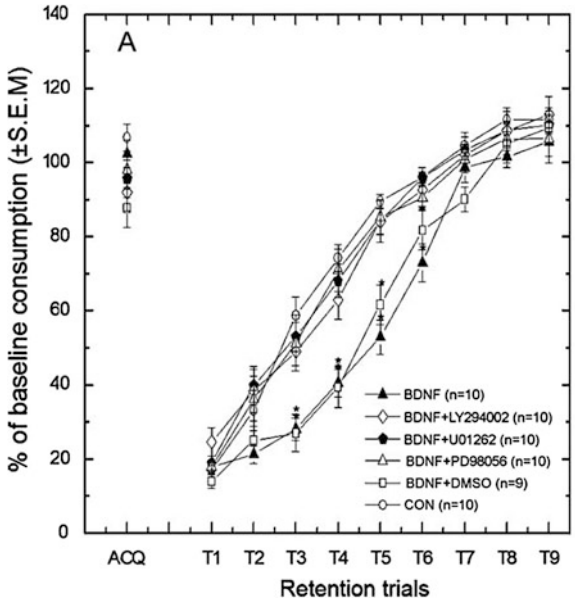

Fig. 5 Mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI-3 K) are required for the modification of conditioned taste aversion (CTA) retention caused by brain-derived neurotrophic factor (BDNF). **a** Point plot of the acquisition session (ACQ) and nine drinking test (T1–T9; one every other day) given to BDNF, BDNF + LY294002 (PI-3 K inhibitor), BDNF + PD98059 (MAPK inhibitor), BDNF + U0126 (MAPK inhibitor), BDNF + DMSO (used as vehicle), and CON (control) groups. **b** Three additional groups receiving LY294002, U0126, and PD98056 in absence of BDNF were trained in the CTA task, showing that the inhibitors themselves did not affect the CTA retention. *$p < 0.001$ (Castillo and Escobar 2011)

2007). Recently, Alonso and colleagues (2002) reported that the presence of BDNF in the hippocampus is essential for the adequate performance of short-term, as well as long-term, memory tasks. Intrahippocampal administration of BDNF increased spatial memory (Cirulli et al. 2004), whereas the administration of antisense oligonucleotides of BDNF reduced retention and inhibited the induction of LTP in the hippocampus of adult rats (Ma et al. 1998). Similarly, two research groups observed that the production of BDNF and its receptor TrkB played a critical role in fear conditioning (Liu et al. 2004; Rattiner et al. 2004). In this sense, recent research conducted in our laboratory shows that intracortical microinfusion of BDNF (at concentrations that produce increases in synaptic efficiency) enhanced the retention of conditioned taste aversion (Castillo et al. 2006; Castillo and Escobar 2011) (see Fig. 5). Furthermore, another study performed in our laboratory showed that BDNF was capable of restoring the aversive memories, previously blocked by the protein synthesis inhibitor anisomycin (Moguel-Gonzalez et al. 2008).

These series of findings support the idea that BDNF plays an important role in information storage within mammalian brains. Although there is very little evidence regarding the role of NT-3 in learning and memory processes, one study shows that mice lacking the NT-3 gene presented reduced neurogenesis in the dentate gyrus, which correlated with deficits in spatial memory tasks (Shimazu et al. 2006). This study points to a potential role of this neurotrophin on hippocampal-dependent memory that requires further study and understanding.

7 Neurotrophins on Metaplasticity

It is widely accepted that learning and memory are dependent on changes in synaptic efficiency and morphology that permit the strengthening of synapses. Thus, the potential of the neurotrophic factors (specifically of BDNF and NT-3) to modulate synaptic transmission and the structural changes resulting from such functional modifications provide valuable tools for dissecting the cellular events involved in the acquisition and storage of information.

In recent years, research on the role of these neurotrophins in long-term synaptic plasticity has experienced rapid growth. However, several key issues still remain unresolved regarding the bidirectional capacity of BDNF and NT-3 to signal glutamatergic synapses.

The involvement of both neurotrophins in so-called synaptic consolidation, through their effects on gene expression and local protein synthesis, are also important questions that remain unresolved.

Experiments indicate the primary involvement of BDNF and NT-3 during the late phase of LTP (Schinder et al. 2000; Poo 2001; Lu et al. 2005). Because the structural changes are considered as key elements of the permanence of synaptic plasticity, the potential for both neurotrophins to promote changes of this nature seem to constitute one of their key mechanisms for information storage.

A series of recent studies have shown that the activity history of a given neuron or pathway modifies its ability to generate subsequent synaptic plasticity (Abraham 2008). In this regard, it has been proposed that homeostatic forms of plasticity might provide the global regulation necessary to maintain synaptic strength and plasticity within a functional dynamic range. These forms of plasticity also operate with diverse mechanisms; for example, by altering the ability of synapses to undergo subsequent Hebbian modifications (metaplasticity), thought to be essential not only to maintain synapses within a dynamic functional range but also to be substantial for the maintenance of a memory trace.

Metaplasticity can significantly modify the susceptibility of synapses for the subsequent induction of plasticity and preset the direction of the future plasticity at these synapses (Abbott and Nelson 2000; Abraham 2008; Nelson and Turrigiano 2008). Recent studies conducted by our group have shown that BDNF and NT-3 initiate the regulatory mechanisms that modify the ability of the mossy fiber pathway to undergo LTP induced by HFS [Schjetnan and Escobar 2012; Ramos-

Languren and Escobar (submitted)]. Such a homeostatic mechanism is thought to be essential for the synaptic integration among prolonged temporal domains in the adult mammalian brain (Abraham 2008). Thus, the role of neurotrophic factors in such expressions of plasticity needs further evaluation.

8 Perspectives

The effects of neurotrophins on the functional and morphological plasticity of synapses have opened an extensive line of research regarding the involvement of these proteins in neurological disorders and injuries. These include: pain, epilepsy, Alzheimer's disease, Parkinson disease, schizophrenia, depression, stroke, and spinal cord injury (Binder and Scharfman 2004; Gu et al. 2009; Im et al. 2010; Garcia-Alias et al. 2011). This line of research has made important advances on identifying factors that involve the regulation of such neurotrophins. For example it has been observed that antidepressant drugs can result in an increase of hippocampal BDNF, resulting in an enhancement of newly generated cells that would improve some of the cognitive impairments associated with depression (Mattson et al. 2004; Banasr and Duman 2008). A recent study suggests that progenitor cells that are rich in NT-3 secretion are more prone to differentiate and survive once transplanted into 6-hydroxydopamine (6-OHDA)-treated Parkinsonian rats (Gu et al. 2009). Furthermore, a recent study supports the idea that secretion of NT-3 promotes axonal plasticity and functional recovery in rats with lateral hemisection of the spinal cord (Garcia-Alias et al. 2011). Finally, transcranial-direct current stimulation (tDCS), that has emerged as a potentially important rehabilitation therapy for disorders such as stroke and depression, has been shown to elicit its effects through BDNF activation (Floel and Cohen 2010; Fritsch et al. 2010).

In summary, the findings reported to date implicate BDNF and NT-3 as key molecular mediators of synaptic plasticity. Both neurotrophins are closely associated with information storage in the adult mammalian brain and its research could enhance the understanding of several neurological diseases and might have a participation in the discovery of new therapeutic strategies.

Acknowledgments This work was supported by PAPIIT IN213210 and CONACYT 60851. We acknowledge the assistance of Zak Stinson, Amanda Mauthe-Kaddoura, and Simone Cherry-Delisle during the preparation of the manuscript.

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Part II
Environmental Factors

Differential Effects of Stress and Glucocorticoids on Adult Neurogenesis

Timothy J. Schoenfeld and Elizabeth Gould

Abstract Stress is known to inhibit neuronal growth in the hippocampus. In addition to reducing the size and complexity of the dendritic tree, stress and elevated glucocorticoid levels are known to inhibit adult neurogenesis. Despite the negative effects of stress hormones on progenitor cell proliferation in the hippocampus, some experiences which produce robust increases in glucocorticoid levels actually promote neuronal growth. These experiences, including running, mating, enriched environment living, and intracranial self-stimulation, all share in common a strong hedonic component. Taken together, the findings suggest that rewarding experiences buffer progenitor cells in the dentate gyrus from the negative effects of elevated stress hormones. This chapter considers the evidence that stress and glucocorticoids inhibit neuronal growth along with the paradoxical findings of enhanced neuronal growth under rewarding conditions with a view toward understanding the underlying biological mechanisms.

Keywords Stress · Neurogenesis · Dentate gyrus · Reward · Learning · Anxiety · Physical activity · Sexual experience

Contents

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Curr Topics Behav Neurosci (2013) 15: 139–164
DOI: 10.1007/7854_2012_233

Published Online: 31 January 2013

1 Introduction

"Stress" has been classically defined as an environmental challenge that produces a physiological response resulting in the release of specific "stress" hormones such as glucocorticoids (McEwen 2002; Sapolsky 2004). Although this classic definition seems neutral, another implicit aspect of most definitions of "stress" is that the environmental challenge has an aversive component. Although neutral or even appetitive stimuli can activate stress hormone systems, the most common contemporary definition of stress connotes a negative state.

It is now generally accepted that stress has detrimental actions on the structure and function of the hippocampus (reviewed in Conrad 2010). Stress is known to alter hippocampal structure and synaptic plasticity in a variety of ways, including decreasing dendritic arborization and spine density (Watanabe et al. 1992; Magariños et al. 1996; McKittrick et al. 2000; Bessa et al. 2009; Christian et al. 2011), decreasing cell proliferation and adult neurogenesis (Gould et al. 1997; Pham et al. 2003; Ferragud et al. 2010), reducing overall hippocampal volume (Golub et al. 2011; Pham et al. 2003), and reducing hippocampal LTP (Foy et al. 1987; Shors et al. 1997; Pavlides et al. 2002), although this latter effect seems to be specific to dorsal subsections of the hippocampus (Maggio and Segal 2007). All of these alterations have been proposed as mediators of stress-induced impairments in hippocampal-dependent learning (Conrad 2006; Howland and Wang 2008; Leuner and Gould 2010). However, more recent work demonstrating either no effects or even positive effects of elevated stress hormones on hippocampal structure and function suggests a broader view is necessary. This review will focus on both negative and positive effects of stress on adult neurogenesis, various modulators of these effects, and functional relevance of changes in hippocampal structure, with an emphasis on adult neurogenesis.

1.1 Adult Neurogenesis in the Mammalian Brain

Because the production of new neurons leads to synaptogenesis, as well as axonal elongation and dendritic elaboration, adult neurogenesis is perhaps the most fundamental of all types of structural change. The rate of adult neurogenesis in the mammalian brain is highest in two regions; the subgranular zone (SGZ), of the dentate gyrus, and subventricular zone (SVZ), from which new neurons migrate to the olfactory bulb (see Sects. 2.1 and 2.2 of this edition). Adult neurogenesis can be divided into three distinct stages: cell proliferation, neuronal differentiation, and cell survival (Christie and Cameron 2006). Each stage represents a plastic process that can be influenced by stress.

Cell proliferation refers to the mitosis of progenitor cells located in the SGZ of the dentate gyrus and the SVZ. Neuronal differentiation refers to the development of daughter cells into neurons. Most new cells in the dentate gyrus differentiate into granule neurons (80–95 %, depending on factors such as species, age, and location of granule cells within the dentate gyrus (Cameron et al. 1993b; Cameron and McKay 2001; Brown et al. 2003; Snyder et al. 2009a). A smaller percentage of new cells ($\sim$10), become glia (Cameron et al. 1993b; Steiner et al. 2004). In the SVZ, new cells migrate along the rostral migratory stream (Luskin 1993; Lois and Alvarez-Buylla 1994) where $\sim$95 % differentiate into granule cells (Lledo and Saghatelyan 2005) with the rest becoming periglomerular cells. The time course of maturation of new cells into neurons in the dentate gyrus can vary, as research has shown that new cells in the adult rat dentate gyrus select a neuronal fate more quickly than those in the adult mouse (Snyder et al. 2009a).

New neurons undergo morphological and electrophysiological alterations as they mature. By a few weeks after cell proliferation, new neurons develop the morphological characteristics of granule cells. New granule cells in the dentate gyrus grow dendritic arbors extending toward the molecular layer (Ribak et al. 2004), send axons into the CA3 region of the hippocampus (Hastings and Gould 1999; Zhao et al. 2006), generate action potentials (van Praag et al. 2002), and are activated by functionally relevant cues (Ramirez-Amaya et al. 2006; Tashiro et al. 2007; Snyder et al. 2012). In the olfactory bulb, new granule cells exhibit dynamic dendritic growth and structural plasticity (Petreanu and Alvarez-Buylla 2002). By the time of their full maturation, new granule cells are electrophysiologically identical to granule cells generated during development (Petreanu and Alvarez-Buylla 2002), and are activated by functionally relevant olfactory cues (Magavi et al. 2005). New granule cells can survive for periods up to a year or longer (Petreanu and Alvarez-Buylla 2002; Dayer et al. 2003), but many new neurons die within a few weeks of their production. In rodents, only about 50 % of new granule cells survive after the first few weeks in the dentate gyrus and olfactory bulb (Petreanu and Alvarez-Buylla 2002; Winner et al. 2002; Dayer et al. 2003). Research has shown that cell proliferation, neuronal differentiation, and cell survival are influenced by multiple environmental factors (Leuner and Gould 2010), with a large number of stress studies examining effects on cell proliferation.

Research has also preferentially focused on environmental influences on adult neurogenesis in the dentate gyrus with fewer studies focused on the SVZ and olfactory bulb.

2 Stress Inhibits Adult Neurogenesis in the Dentate Gyrus

2.1 Acute Stress Reduces Cell Proliferation

Overall, research suggests that acute exposure to a stressful situation decreases cell proliferation in the dentate gyrus. Acute exposure to a dominant conspecific (social defeat) reduces cell proliferation in the dentate gyrus in mice, tree shrews, and marmosets (Gould et al. 1997, 1998; Yap et al. 2006; Lagace et al. 2010). Similarly, acute exposure to trimethylthiazoline, a natural odor of foxes, predators to rodents, decreases cell proliferation in the rat (Tanapat et al. 2001; Mirescu et al. 2004; Hill et al. 2006; Kambo and Galea 2006). Moreover acute electric shock decreases cell proliferation in the rat (Malberg and Duman 2003). In addition to studies examining cell proliferation, decreases in neuronal differentiation are seen following acute predator odor in the rat (Tanapat et al. 2001), and decreased survival of new granule cells has been observed following acute social defeat and acute predator odor exposure in the rat (Tanapat et al. 2001; Thomas et al. 2007).

The effects of acute physical restraint stress are less clear than the results from the stressors already discussed. Multiple studies show that acute restraint stress lasting 2–6 h does not change cell proliferation in adult rats (Kee et al. 2002; Pham et al. 2003; Rosenbrock et al. 2005). However, one study has shown that 3 h of restraint decreases cell proliferation in the adult rat, yet increases cell proliferation in adult mice (Bain et al. 2004). Because variations in restraint protocols are likely to differentially affect the stress response; comparisons among different restraint studies are often difficult to make (Buynitsky and Mostofsky 2009).

2.2 Chronic Stress Reduces Cell Proliferation, Neuronal Differentiation, and Cell Survival

Chronic stress paradigms normally involve stress induction over the course of days to weeks. Chronic social defeat decreases cell proliferation in the dentate gyrus of tree shrews (Czeh et al. 2001, 2002; Simon et al. 2005), rats (Czeh et al. 2007), and mice (Mitra et al. 2006; Ferragud et al. 2010). In mice, decreases in cell proliferation actually correlate with typical behavior of subordinates (Mitra et al. 2006). Chronic social defeat also decreases the differentiation of new neurons in rats and mice (Ferragud et al. 2010; Van Bokhoven et al. 2011) and reduces the survival of new neurons in tree shrews (Czeh et al. 2002) and rats (Czeh et al. 2007). Similarly, chronic electric shock decreases cell proliferation, neuronal differentiation,

and cell survival in rats (Westenbroek et al. 2004; Dagyte et al. 2009). Chronic restraint stress studies show similar discrepancies as reported above for acute restraint stress studies. Chronic restraint stress has been reported to decrease cell proliferation, neuronal differentiation, and cell survival in rats (Pham et al. 2003; Veena et al. 2011a, b), have no effect on cell proliferation in rats (Rosenbrock et al. 2005; Barha et al. 2011), and even increase survival of new neurons in rats and mice (Snyder et al. 2009b; Barha et al. 2011). Again, differences in age, strain, housing conditions, and type, duration, and frequency of restraint may explain differences in the effect of chronic restraint stress on adult neurogenesis in the dentate gyrus.

Numerous studies indicate that training on various learning paradigms stimulates adult neurogenesis in rats (Gould et al. 1999; Leuner et al. 2004, 2006; Epp et al. 2010). However, when learning is complex or prolonged, it appears to decrease cell proliferation (Aztiria et al. 2007; Dupret et al. 2007). Stress-related novelty in a testing environment decreases cell proliferation even though learning occurs (Ehninger and Kempermann 2006). Finally, increased task difficulty does not affect cell proliferation, but cell survival decreases in a step-wise manner (Epp et al. 2010).

Chronic exposure to multiple mild stressors can serve as an animal model depression, as animals can develop symptoms of learned helplessness over the course of days and weeks. Switching the stressors during the experiment prevents habituation. Stressors commonly used in these types of studies vary greatly and include cold-water swim, immobilization, social isolation, food and water deprivation, chronic illumination, white noise exposure, tail pinch, tilted or shaken cage, and electric shock, although experiments typically do not use all of the above. It should be noted that the specific types of stressors used may be responsible for producing differential effects on adult neurogenesis. In general, cell proliferation is decreased following exposure to multiple stressors (Xu et al. 2007; Surget et al. 2008). Some studies suggest that this effect may be limited to ventral portions of the hippocampus (Elizalde et al. 2010; Tanti et al. 2012), although most earlier studies on this topic did not differentiate between dorsal and ventral parts of the hippocampus. Stress can also diminish differentiation and survival of neurons born before stressor exposure (Lee et al. 2006; Oomen et al. 2007; Dagyte et al. 2011). Together, these studies show that chronic exposure to stressful situations is detrimental to adult neurogenesis in the dentate gyrus in that it decreases cell proliferation, neuronal differentiation, and cell survival.

3 Variations in Age and Sex Complicate the Effects of Stress on Adult Neurogenesis in the Dentate Gyrus

The rates of cell proliferation and adult neurogenesis decrease with age in the dentate gyrus of all species examined, including mice, rats, tree shrews, dogs, marmosets, and macaques (Seki and Arai 1995; Kuhn et al. 1996; Cameron and

McKay 1999; Gould et al. 1999; Simon et al. 2005; Leuner et al. 2007). Stress produces a greater decrease in cell proliferation in the aged tree shrew compared to younger adult tree shrews (Simon et al. 2005), suggesting that older animals may be more susceptible to the negative effects of stress.

Sex differences in baseline adult neurogenesis in the dentate gyrus in control animals have not been reported, although cell proliferation rates vary due to the phase of estrous cycle in female rats (Tanapat et al. 1999), but not in female mice (Lagace et al. 2007). However, females and males have shown differences in how stress affects the production of new neurons. The reduction in cell proliferation in adult male rats after exposure to predator odor is not seen in female rats (Falconer and Galea 2003). Male rats show decreases in the survival of new neurons following chronic electric shock, but increases are seen in female rats (Westenbroek et al. 2004). A recent study suggests that female rats have decreased cell proliferation and survival following chronic restraint, but male rats show no change in cell proliferation, but an increase in cell survival (Barha et al. 2011). These results suggest that male and female animals may respond to stress differently although a clear picture has yet to emerge from these studies.

It should be noted that new neurons in the dentate gyrus mature along a different time scale in adult rats and adult mice. Snyder et al. (2009a) discovered that rats produce a higher number of new cells, and that these new cells mature faster, and show greater activation to functional stimuli than such cells in the mouse. Therefore, potential differences between rats and mice on effects of stress on adult neurogenesis must take into account the inherent differences that exist between rats and mice in baseline conditions.

4 Effects of Stress on Cell production in the SVZ

Research on the effects of various stressors on adult neurogenesis in the SVZ has been limited. Chronic restraint stress has been shown to decrease survival of new neurons in the olfactory bulb, but has no effect on cell proliferation in the SVZ (Kaneko et al. 2006). Chronic forced swim stress decreases the number of progenitor cells in the SVZ (Hitoshi et al. 2007). Chronic exposure to multiple mild stressors reduces the number of immature neurons in the SVZ, although this was measured using an endogenous marker of immature neurons, so it is unclear whether these effects are from decreased proliferation or differentiation into new neurons (Yang et al. 2011). Because chronic exposure to mild multiple stressors does not affect cell proliferation in the SVZ (Silva et al. 2008), the effect from Yang et al. (2011) may be a result of decreased differentiation of new cells into neurons. Conversely, chronic exposure to mild multiple stressors reduces the number of proliferating cells in the SVZ, but it is unclear whether this reflects on decreases in the number of progenitor cells or decreases in the rate of cell proliferation (Mineur et al. 2007).

5 Rewarding Experiences Enhance Adult Neurogenesis in the Dentate Gyrus, Despite Elevated Levels of Stress Hormones

Despite the numerous studies linking stress and elevated glucocorticoid levels to suppressed neurogenesis, there are some experiences that stimulate the release of stress hormones, but enhance adult neurogenesis in the dentate gyrus. For example, running increases stress hormone, or glucocorticoid, levels in the blood (Droste et al. 2003; Makatsori et al. 2003; Stranahan et al. 2006), yet increases cell proliferation, induces neuronal differentiation, and enhances survival of new neurons in the dentate gyrus of both mice (van Praag et al. 1999; Klaus et al. 2009; Snyder et al. 2009b) and rats (Stranahan et al. 2006; Yi et al. 2009). Alcohol-induced impairments in cell proliferation can be rescued by running (Crews et al. 2004). This suggests that running engages mechanisms that protect progenitor cells or new neurons from the detrimental effects of stress-induced release of glucocorticoids. However, running does not change proliferation of new cells in the SVZ or the number of new neurons in the olfactory bulb (Brown et al. 2003; Crews et al. 2004; Schoenfeld et al., unpublished observations). Similarly, housing in an enriched environment can elevate glucocorticoid levels (Benaroya-Milshtein et al. 2004), while increasing neuronal differentiation and cell survival in adult and aged mice (van Praag et al. 1999; Kempermann et al. 2002). Living in an enriched environment also ameliorates stress-induced reductions in cell proliferation, neuronal differentiation, and cell survival in the adult rat (Veena et al. 2009a, b). Again, this suggests some protective mechanism of enriched environment living that allows for neuronal growth despite elevated glucocorticoid levels. However, as observed with running, environmental enrichment has no effect on proliferation of new cells in the SVZ or the number of new neurons in the olfactory bulb (Brown et al. 2003; Plane et al. 2008).

Sexual experience also increases circulating glucocorticoid levels (Bonilla-Jaime et al. 2006). Both acute and repeated sexual experiences increase cell proliferation in the dentate gyrus of adult rats, and chronic sexual experience also enhances survival of new neurons in the dentate gyrus (Leuner et al. 2010). The effect of sexual experience on cell proliferation and neurogenesis in the olfactory bulb has not been examined.

Taken in the context of the negative actions of most stressors on adult neurogenesis, the findings on the positive effects of running, enriched housing, sexual experience, and learning (Leuner and Gould 2010) raise the question of whether these experiences share commonalities that permit neuronal growth despite increased glucocorticoid levels. In this regard, it may be relevant that all of these experiences are rewarding. Rats show anticipatory behavior toward gaining access to an enriched environment (van der Harst et al. 2003). Rats form place preferences for running wheels and mating chambers (Belke and Wagner 2005; Tenk et al. 2009) and can be trained readily to bar press to gain access to wheels or receptive females (Hundt and Premack 1963; Everitt et al. 1987). Intercranial self-

stimulation, a rewarding laboratory experience that taps into circuitry likely engaged in natural reward, results in increased cell proliferation in the dentate gyrus of adult rats and mice as well as elevated glucocorticoid levels (Takahashi et al. 2009). Taken together, these findings suggest that rewarding experience may encourage mechanisms that protect the brain from negative influences of glucocorticoids.

6 Mechanisms of Adult Neurogenesis Inhibition and Stimulation

6.1 Adrenal Steroids

By definition, stress activates the hypothalamic-pituitary-adrenal (HPA) axis, which results in an elevation of glucocorticoids in the blood. Exogenous administration of corticosterone, the main rodent glucocorticoid, results in a decrease in cell proliferation and survival in both the dentate gyrus and SVZ (Cameron and Gould 1994; Wong and Herbert 2006; Lau et al. 2007; Brummelte and Galea 2010a). The inhibition of cell proliferation by corticosterone occurs in both males and females (Brummelte and Galea 2010a) and appears to be independent of reproductive status (Brummelte and Galea 2010b). Conversely, removal of circulating glucocorticoids by adrenalectomy (ADX) promotes cell proliferation and adult neurogenesis in the dentate gyrus (Gould et al. 1992; Cameron and Gould 1994) and SVZ (Guo et al. 2010). Taken together, these findings suggest that the rate of cell proliferation and adult neurogenesis in the dentate gyrus and SVZ of adult rodents can be moderated by circulating levels of glucocorticoids. Since corticosterone injections produce similar effects on adult neurogenesis as stress, it is likely that the stress-induced increases in glucocorticoid levels are responsible for the stress-induced decreases in adult neurogenesis. Indeed, inhibitory effects of fox odor exposure on cell proliferation in the dentate gyrus can be blocked by preventing the stress-induced rise in glucocorticoids (Tanapat et al. 2001). It remains unknown, however, whether these effects are mediated directly via actions of adrenal steroids on progenitor cells or whether they occur indirectly through some unknown factor.

Glucocorticoids bind to two main types of receptors in the brain, the glucocorticoid receptor (GR) and the mineralocorticoid receptor (MR) (Reul and de Kloet 1985). Granule cells in the dentate gyrus and olfactory bulb express both types of adrenal steroid receptors (Morimoto et al. 1996). Because, MRs have higher affinity for glucocorticoids than GRs, MRs are more likely to be sensitive to circadian changes in glucocorticoid levels, while GRs are more likely to respond to stress-induced elevations in glucocorticoid levels (de Kloet et al. 1998). Direct activation of MRs through the MR-agonist aldosterone in adult ADX rats enhances cell proliferation and neurogenesis (Fischer et al. 2002), while activation of GRs

through the GR-agonist dexamethasone in adult rats inhibits cell proliferation (Kim et al. 2004), further suggesting that elevation of stress hormones act through GRs to reduce hippocampal neurogenesis.

Although most new neurons express both GR and MR after 4 weeks of maturation, relatively few progenitor cells express adrenal steroid receptors (Cameron et al. 1993a; Garcia et al. 2004). This raises the possibility that adrenal steroid-mediated changes in the rate of cell proliferation in the dentate gyrus occur indirectly. There are several possible mechanisms whereby such an indirect effect might occur. For instance, glucocorticoids might affect neurogenesis by influencing neighboring, more mature, granule neurons. This could occur either by altering the survival of granule cells directly or by affecting their afferent inputs.

With regard to the first possibility, ADX results in massive death of mature granule cells in the dentate gyrus (Sloviter et al. 1989; Gould et al. 1990). Replacement of ADX rats with aldosterone, a mineralocorticoid that binds with high affinity to MRs, is sufficient to protect the dentate gyrus from cell death (Woolley et al. 1991), suggesting that regular activation of MRs is important for the maintenance of the granule cell population. These findings suggest that dying mature granule cells may provide signals that stimulate the proliferation of progenitor cells. In this regard, it is relevant to note that direct destruction of the dentate gyrus, via chemical or mechanical lesion, leads to an increase in the production of new neurons (Gould and Tanapat 1997). The link between cell survival and cell proliferation has not been extensively explored in the dentate gyrus, but several reports suggest that neuronal death can stimulate adult neurogenesis in many other brain regions, including the neocortex and striatum (Gould 2007).

An additional, but not mutually exclusive, possibility is that adult neurogenesis is affected indirectly through adrenal steroid actions on granule cell afferents. Lesion of the entorhinal cortex, one of the main afferent populations to the dentate gyrus, stimulates the production of new neurons (Cameron et al. 1995). Likewise, blockade of NMDA receptors, glutamate receptors involved in perforant path-granule cell synapses, increases adult neurogenesis (Cameron et al. 1995; Maekawa et al. 2009). Moreover, manipulation of cholinergic inputs, via either neurotoxin or pharmacological intervention, alters the rate of adult neurogenesis (Kotani et al. 2006; Frechette et al. 2009). Although not directly explored in the context of adrenal steroids, these afferent populations contain adrenal steroid receptors, and may be one of the intermediate steps between alterations in hormone levels and changes in the production of new neurons.

6.2 Cytokines, Neurotrophins and Neuropeptides

Inflammation decreases cell proliferation in the rodent dentate gyrus (Ekdahl et al. 2003; Monje et al. 2003). Interleukin-1 (IL-1) is a proinflammatory cytokine that is a member of a family of immune factors that communicate inflammation to the

central nervous system. IL-1 incites glucocorticoid release by the adrenal glands (Bernton et al. 1987). Therefore, IL-1 has been implicated in moderating the negative effects of stress on cell proliferation and neurogenesis. Exogenous administration of IL-1β inhibits cell proliferation and neuronal differentiation in the dentate gyrus of adult mice (Goshen et al. 2008; Koo and Duman 2008). Progenitor cells in the SGZ have IL-1 receptors, which decrease cell proliferation when activated (Koo and Duman 2008). Inactivation of IL-1 receptors prevents stress-induced decreases in cell proliferation (Goshen et al. 2008; Ben Menachem-Zidon et al. 2008) suggesting that inflammatory cytokines may also regulate adult neurogenesis. Interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) are also proinflammatory cytokines associated with mediating adult neurogenesis. Adult IL-6 knockout mice show enhanced proliferation and survival of new neurons in the dentate gyrus and SVZ (Bowen et al. 2011) and adult selective TNF receptor knockout mice have promoted neurogenesis in the dentate gyrus (Iosif et al. 2006). Also overproduction of IL-6 impairs adult neurogenesis in the dentate gyrus (Vallieres et al. 2002), further suggesting that inflammatory cytokines can decrease cell proliferation and may be involved in stress-induced deficits in adult neurogenesis. Other inflammatory cytokines are likely to moderate the effects of stress on neurogenesis in the adult.

Neurotrophic factors are important in regulating embryonic neuronal development, and many of these factors are altered after exposure to rewarding experiences that promote adult neurogenesis. Brain-derived neurotrophic factor (BDNF) is a factor in the survival of new neurons in the dentate gyrus (Sairanen et al. 2005), and blocking BDNF decreases the differentiation of new neurons in adult mice (Taliaz et al. 2010). BDNF is released following running (Ying et al. 2005), and is required for enriched environment-induced increases in cell proliferation (Rossi et al. 2006). Vascular endothelial growth factor (VEGF) promotes cell proliferation in the adult rat dentate gyrus (Jin et al. 2002). Chronic stress decreases VEGF expression (Heine et al. 2005). VEGF is necessary for increases in cell proliferation and neuronal differentiation in running mice (Fabel et al. 2003), and for enriched environment-induced cell proliferation, neuronal differentiation, and cell survival in rats (Cao et al. 2004). Hence, VEGF is another potential factor in moderating adult neurogenesis. Insulin-like growth factor 1 (IGF-1) increases cell proliferation in the adult rat dentate gyrus (Aberg et al. 2000) and moderates other positive effects in the brain after running (Carro et al. 2000). Hence, IGF-1 may also be a factor in promoting adult neurogenesis, although research has not investigated the specific role of IGF-1 in the positive neurogenic aspects of reward experiences. No studies have yet examined the roles of BDNF, VEGF, or IGF-1 in sexual experience. Although rewarding behaviors such as running and environmental enrichment do not affect proliferation and neurogenesis in the SVZ, BDNF and VEGF administration also increase proliferation of cells in the SVZ and the number of new neurons in the olfactory bulb (Zigova et al. 1998; Jin et al. 2002; Sun et al. 2006), so growth factors may prevent decreases in SVZ neurogenesis from elevated glucocorticoid levels.

The neuropeptide oxytocin is also worth considering in the context of the effects of rewarding experience on adult neurogenesis. Oxytocin is released in the hippocampus under conditions of social reward, such as during mating (Waldherr and Neumann 2007), and has been shown to buffer against the negative actions of stress (Windle et al. 2004, 2006). Oxytocin has also been shown to stimulate cell proliferation and adult neurogenesis in the dentate gyrus, even under conditions of elevated glucocorticoids and stress (Leuner et al. 2012). These findings raise the possibility that under conditions of reward, oxytocin release into the hippocampus may bypass the suppressant actions of glucocorticoids on progenitor cells. No studies have yet addressed this possibility directly.

Neuropeptide Y (NPY) is another candidate in moderating the effects of rewarding experience on adult neurogenesis. NPY is upregulated in the dentate gyrus of adult mice during running (Bjornebekk et al. 2006), and administration of NPY stimulates adult neurogenesis in the dentate gyrus (Howell et al. 2003, 2005). Because NPY has been associated with behavioral resiliency to stressors (Thorsell et al. 2000; Carvajal et al. 2004; Cohen et al. 2012), it may mediate the positive effects of certain rewarding stressors on adult neurogenesis.

Given that corticotropin releasing factor (CRF) and vasopressin are important modulators of the HPA axis (Holsboer 1999; Aguilera and Rabadan-Diehl 2000), both neuropeptides may be involved in stress-induced decreases in cell proliferation and neurogenesis. Although peripheral injections of vasopressin have not been shown to affect rates of cell proliferation in the dentate gyrus (Leuner et al. 2012), selective vasopressin and CRF receptor antagonists have been shown to reverse the impairment of cell proliferation and neurogenesis in the dentate gyrus of chronically stressed mice (Alonso et al. 2004), suggesting that the neuropeptides CRF and vasopressin may play a role in stress-induced changes in adult neurogenesis, although they may act indirectly through manipulation of the HPA axis.

6.3 Neurotransmitters

Neurotransmitters affect new neuron production and may be involved in the positive and negative effects of different stressors on adult neurogenesis. Excitatory neurotransmitters, such as glutamate, have been shown to have suppressive effects on adult neurogenesis in the dentate gyrus. Activation of glutamatergic NMDA receptors decreases cell proliferation and survival in the adult dentate gyrus, while the use of NMDA antagonists shows the opposite action (Cameron et al. 1995; Gould et al. 1997; Nacher et al. 2003). Consistent with studies on NMDA receptor blockade, lesion of the entorhinal cortex, a major source of glutamatergic input to the granule cells through the perforant path has been shown to increase adult neurogenesis in the dentate gyrus (Cameron et al. 1995). Somewhat surprisingly, a recent study has shown that electrical stimulation of the entorhinal cortex also stimulates adult neurogenesis (Stone et al. 2011) raising the possibility that naturally occurring patterns of entorhinal input typically dampen

new neuron production. Disruption of this pattern, either through removal of the afferent population or artificial electrical stimulation removes this brake and allows a higher rate of adult neurogenesis.

Given the effects that rewarding experiences have on adult neurogenesis, it is perhaps unsurprising that dopamine has been implicated in the regulation of adult neurogenesis. However, mixed results have been reported showing either transient increases or decreases in cell proliferation following dopamine depletion (Hoglinger et al. 2004; Park and Enikolopov 2010). The effect of dopamine on cell proliferation may be receptor-dependent (Veena et al. 2011a, b), and importantly, recent evidence has shown that the new neurons respond differently to dopaminergic activation than mature granule cells, suggesting that dopamine may have a specific role in the maturation and integration of proliferated neurons (Mu et al. 2011). The neurotransmitter serotonin has been shown to also have positive effects on cell proliferation and neurogenesis in the adult. Ablation of serotonin innervation into the dentate gyrus and serotonin receptor antagonists decreases cell proliferation (Brezun and Daszta 2000; Radley and Jacobs 2002). Importantly, the use of antidepressants which selectively block serotonin reuptake can reverse stress-induced decreases in cell proliferation (Qiu et al. 2007; Hitoshi et al. 2007), suggesting that the actions of positive stressors on adult neurogenesis may work as well through serotonergic mechanisms. These neurotransmitters have also been seen to have similar effects on neurogenesis in the SVZ (reviewed in Young et al. 2011), suggesting that common mechanisms may underlie these effects in both regions.

Numerous studies suggest that GABA also plays an important role in adult neurogenesis. In the dentate gyrus, both progenitor cells and new neuroblasts contain functional GABA-A receptors (Wang et al. 2005). Manipulating these receptors alters proliferation of progenitor cells in the SGZ, as administration of GABA-A agonists decrease cell proliferation, and GABA-A antagonists increase cell proliferation (Tokuza et al. 2005). During the early stage of maturation, new cells respond to GABA with excitatory actions (Espósito et al. 2005; Overstreet Wadiche et al. 2005; Ge et al. 2006). New neurons have immature Cl^- channels that cause GABA to have a depolarizing effect during the first few weeks of maturation (Ge et al. 2006; Pathania et al. 2010). This GABAergic depolarization of immature granule cells appears to be important for dendritic growth and neuronal differentiation (Deisseroth et al. 2004; Ge et al. 2006). Blocking GABAergic transmission in immature neurons causes decreased spine density and shorter dendrites upon maturation (Sun et al. 2009). Ge et al. (2006) showed that altering Cl^- channels to make GABA hyperpolarizing in immature neurons truncates dendritic growth and synapse formation. Once new neurons mature, they show typical GABAergic hyperpolarization, and enhanced synaptic potentiation, compared to preexisting neurons (Ge et al. 2006). Interestingly, even after GABA becomes hyperpolarized, new neurons exhibit enhanced LTP compared to older neurons (Ge et al. 2007).

Similar effects of GABA on the development and maturation of new neurons in the adult olfactory bulb have been observed. New GABAergic cells in the

olfactory bulb release GABA into the extracellular space which acts on progenitor cells to dampen cell proliferation (Liu et al. 2005). Increased extracellular GABA slows migration of immature neurons to the olfactory bulb (Bolteus and Bordey 2004; Platel et al. 2008). As observed for new neurons in the dentate gyrus, GABA is depolarizing to immature olfactory bulb neurons and enhances the complexity of their dendritic arbors (Gascon et al. 2006). Blocking GABA-A receptors has been shown to decrease dendritic length of new olfactory bulb neurons (Gascon et al. 2006).

Stress and corticosterone increases dampen GABA release in the hippocampus (de Groote and Linthorst 2007; Grønli et al. 2007; Martisova et al. 2012). Therefore, both acute and chronic stress may affect not only the rate of cell proliferation and neurogenesis in the hippocampus, but GABA signaling as well. Decreased GABA signaling in the dentate gyrus due to stress may have detrimental effects on new neuron maturation and dendritic complexity. On the other hand, running alters GABA-A receptor expression in the dentate gyrus (Hill et al. 2010). Although specific expression in new neurons was not measured, it is reasonable to suggest that running also changes GABA-A receptors in new neurons. Running is known to increase expression of GAD67, the synthetic enzyme for GABA, in the dentate gyrus (Hill et al. 2010), and our recent findings demonstrate that runners have increased expression of vGAT in the dentate gyrus and show a transient increase in GABA release in the hippocampus following stress (Schoenfeld et al., unpublished observations). New neurons born during running in the dentate gyrus mature faster than in a control condition (Snyder et al. 2009b). These results suggest that, opposed to stress, running not only increases the production of new granule cells, but also may foster a GABAergic environment that encourages dendritic growth and heightened maturation in new neurons (Fig. 1). It should be noted that prolonged excitation of new neurons through seizure causes aberrant dendritic growth in new neurons (Jessberger et al. 2007), and aberrant development has been linked to psychiatric disorders such as schizophrenia (Lewis and Levitt 2002). However, running has also been shown to ameliorate behavioral deficits in mouse models of schizophrenia (Wolf et al. 2011); hence, increased dendritic growth of new neurons, is not likely to be severe enough to contribute to pathogenesis.

7 Functional Implications of Changing the Rate of Adult Neurogenesis in the Dentate Gyrus

Since new neurons in the dentate gyrus have been shown to generate action potentials and are activated by hippocampal-dependent behaviors (van Praag et al. 1999; Ramirez-Amaya et al. 2006; Kee et al. 2007; Tashiro et al. 2007; Snyder et al. 2009c; Epp et al. 2011; Snyder et al. 2012), it follows that changes in the rate of adult neurogenesis, either inhibiting or enhancing it, will have a consequence on

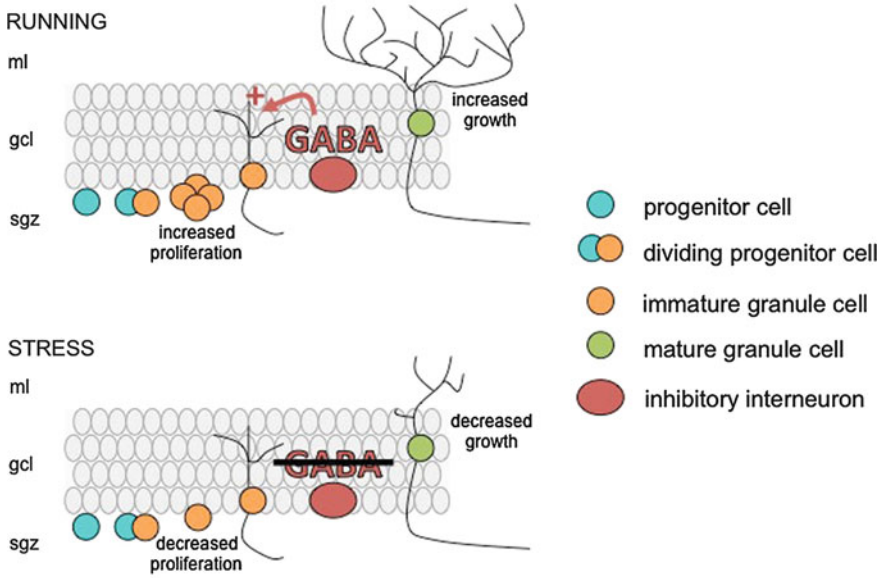

Fig. 1 Schematic diagram of the effects of running and stress on cell proliferation and maturation of new granule cells in the dentate gyrus. Running increases production of new cells and stimulates GABA release. GABA depolarizes immature neurons, fostering dendritic growth and synaptic maturation. Conversely, stress decreases proliferation of new cells and blocks GABA release, resulting in stunted dendritic growth and maturation

hippocampal-dependent functions. The hippocampus contributes to specific types of learning and memory (Moser et al. 1993; Ergorul and Eichenbaum 2004), is important for anxiety regulation (Bannerman et al. 2004; Fanselow and Dong 2010), and modulates feedback of the stress response (Herman et al. 1989; Jacobson and Sapolsky 1991; Herman et al. 1995; Herman and Mueller 2006). All of these functions can be affected by manipulations that are known to change the rate of adult neurogenesis.

Cell proliferation in the dentate gyrus can be knocked down through administration of antiproliferative agents (Shors et al. 2001; Garthe et al. 2009), irradiation (Madsen et al. 2003), and with transgenic models (Garcia et al. 2004). Decreasing neurogenesis in rats results in impaired spatial learning on the Morris water maze, contextual fear conditioning, and trace eye blink conditioning, with no effect on hippocampal-independent cued fear conditioning and delayed eyeblink conditioning (Shors et al. 2001, 2002; Madsen et al. 2003; Snyder et al. 2005; Winocur et al. 2006; Saxe et al. 2006; Warner-Schmidt et al. 2008; Imayoshi et al. 2008; Farioli-Vecchioli et al. 2008; Jessberger et al. 2009). In rats, spatial learning and contextual fear conditioning deficits do not appear until at least 4 weeks following the reduction in new neuron production (Shors et al. 2002; Madsen et al. 2003; Snyder et al. 2005, 2009a; Jessberger et al. 2009), suggesting that the maturation of new neurons is necessary for hippocampal-dependent learning in

rats. In mice, findings are less clear. Studies have shown either deficits or no change in contextual fear conditioning and spatial learning in mice of different strains, sex, and ages, from different time points following ablation (reviewed in Castilla-Ortega et al. 2011). Because new neurons mature more slowly in mice than rats (Snyder et al. 2009a), and different mouse strains exhibit different baseline rates of adult neurogenesis (Kempermann and Gage 2002; Schauwecker 2006; Clark et al. 2011), differences in the effects of deleting adult neurogenesis on learning in mice may be difficult to interpret as general phenomena.

The dentate gyrus has been implicated in mediating pattern separation, the process where highly similar, overlapping representations are dissociated to keep them independent in episodic memory (O'Reilly and McClelland 1994). Adult neurogenesis has been proposed as an important mechanism for pattern separation in the dentate gyrus (Deng et al. 2010). Increasing adult neurogenesis through genetic induction of cell proliferation results in enhanced spatial pattern separation in adult mice (Sahay et al. 2011). Knocking out hippocampal neurogenesis impairs spatial pattern separation in adult mice (Clelland et al. 2009; Tronel et al. 2012). Running-induced increases in cell proliferation are correlated with higher spatial pattern separation in adult mice (Creer et al. 2010). This evidence suggests that stress-reduced adult neurogenesis in the dentate gyrus may have profound effects on hippocampal-dependent memory formation and learning.

Recent evidence indicates that the new neurons play an important role in shutting off the HPA axis after stress (Snyder et al. 2011; Surget et al. 2011). Corticosterone levels are slower to recover to baseline following moderate stress, and the HPA axis is less suppressed by dexamethasone, showing impaired HPA axis feedback, in adult mice without new neurons in the dentate gyrus (Snyder et al. 2011). Although Surget et al. (2011) did not find differences in HPA axis recovery following stress of animals with ablated neurogenesis, they found that the beneficial actions of antidepressants on HPA axis recovery following stress required new neurons. Taken together, these findings suggest that new neurons may play an important role not only in the cognitive functions of the hippocampus, but also in stress regulation. The extent to which stress-induced reductions in adult neurogenesis contribute to increased pathological processes associated with chronic stress, such as anxiety disorders, depression, and HPA axis dysregulation, remains to be determined.

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Effects of Environmental Enrichment Exposure on Synaptic Transmission and Plasticity in the Hippocampus

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Abstract Exposure to an enriched environment (EE) is beneficial to the structure and function of the brain. The added sensory, social, and spatial complexity of the EE also improves cognitive functions such as memory in both healthy brains and damaged or diseased brains, yet the underlying neural mechanisms of these cognitive improvements are poorly understood. In particular, studies that have examined the effects of EE on cellular function in the hippocampus, a structure critical for memory storage, have produced somewhat confusing results. Experiments performed in ex vivo hippocampal slices have reported a variety of EE effects on synaptic transmission and plasticity in both CA1 and the dentate gyrus. However, together with data from in vivo recordings made during and after the EE treatment, the overall results suggest an evolution of changes in neuronal function in the hippocampus, whereby there is an early transient increase in cell activity and plasticity that gives rise to more subtle long-term enhancements in cellular and network function that may contribute to enhanced hippocampus-dependent cognition.

Keywords Enriched environment · Hippocampus · Memory · Synaptic transmission · Synaptic plasticity · Long-term potentiation

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Curr Topics Behav Neurosci (2013) 15: 165–187
DOI: 10.1007/7854_2012_215

Published Online: 15 July 2012

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The structure and function of the brain can be shaped profoundly by experience, which can generate either beneficial or detrimental outcomes depending on the nature of the experience. For example, depriving the brain of normal visual input during early life causes severe detrimental effects on adult vision, whereas increasing or enriching the sensory environment can be beneficial to sensorimotor and cognitive function. Understanding how brain function is improved through enriched environmental (EE) experience is of particular interest. In a time of an aging demographic, people are more prone to aging-related cognitive decline as well as being more likely to suffer from severe impairments of brain function arising from disorders such as stroke and Alzheimer's disease. Enriched environments have been shown to be protective or promote recovery in animal models of many such diseases (Nithianantharajah and Hannan 2006), yet the neural plasticity that occurs during environmental enrichment is poorly understood. In this chapter, we will review the evidence that neural plasticity is induced or altered by enriched environments, focusing on the hippocampus and the spatial memory system it subserves, and will consider the functional implications of such plasticity.

1 Anatomical Changes

The earliest evidence for experience-dependent neural plasticity came from two quite different lines of experiments. Wiesel and Hubel (1963) deprived kittens of normal vision in one eye during the first weeks of life and found that the deprived eye was effectively blinded, never regaining normal vision even after it had be reopened for an extended period of time. Single cell recordings in the primary visual cortex revealed that the deprived eye had become functionally disconnected from the primary visual cortex, exhibiting a drastically reduced ability to drive cortical neurons. Conversely, instead of depriving part of the animal's sensory experience, Rosenzweig et al. (1962) allowed rats to experience an enriched environment, i.e., a much larger housing than the standard laboratory housing and one that also contained conspecifics for social interaction and a variety of objects to provide a rich sensory experience. Their initial report showed that rats that

experienced this EE had heavier brains than regularly housed rats, suggesting that the enriched experience had caused neuroanatomical changes.

The discovery that EE could possibly make a brain 'grow' in some way, was a provocative one and the phenomenon has been studied in much detail over the years. Shortly, after the initial report of an increase in brain weight, it was demonstrated that this effect was most evident in posterior regions of the cortex (Bennett et al. 1964). It was subsequently shown that there was also an increase in cortical thickness (Diamond et al. 1966). When examined on a finer scale, looking at the level of individual neurons, the density of neurons in the cortex was actually reduced in enriched rats (Turner and Greenough 1985). However, this was offset by a larger cell volume, as well as increases in a number of measures of dendritic tree complexity, including dendritic branching, spine density, and synapses per neuron(Volkmar and Greenough 1972; Turner and Greenough 1985; Globus et al. 1973)

Following these initial findings of experience-dependent anatomical plasticity in the neocortex, many experiments on EE effects shifted their focus to the hippocampus. Functionally, the hippocampus sits at an apex of the cortical processing chain, receiving inputs from multiple association areas of neocortex, and in turn sending efferent projections back to the majority of the neocortex (Swanson and Kohler 1986; Van Hoesen 1982). Based on its widespread interaction with the neocortex, it is not surprising that the hippocampus plays a critical role in higher cognitive function. In particular, it has been shown to play an essential role in memory storage ever since the discovery of the profound amnesia that occurs following its removal (Scoville and Milner 1957). Additionally, the discovery of long-term potentiation in the hippocampus launched a significant body of research on hippocampal physiology, providing a foundation of data against which experiments on EE could be compared.

Changes in anatomy in the hippocampus following EE appear to be less robust than those observed in the neocortex. An initial report demonstrated a change in hippocampal thickness, similar to that observed in the neocortex (Walsh et al. 1969). However, this effect could not be replicated (Diamond et al. 1976). Increases in dendritic tree complexity of dentate granule cells have been observed, but only if the EE was started at weaning, and not if it was started in adulthood (Fiala et al. 1978), and may depend on the sex of the animal (Juraska et al. 1985). In CA1, initial reports indicated an increase in spine density following EE which was restricted to basal dendrites (Moser et al. 1994, 1997), but more recent reports have also observed similar increases on apical dendrites (Rampon et al. 2000; Malik and Chattarji 2012). Interestingly, although the evidence for an effect of EE on hippocampal structure is variable, the hippocampus is necessary for the anatomical changes in the neocortex to occur (Sutherland et al. 2010).

One consistent anatomical change in the hippocampus following EE is an increase in neurogenesis in the subgranular zone of the dentate gyrus. This increase was first demonstrated in mice that were exposed to an enriched environment for 40 days (Kempermann et al. 1997). In an attempt to determine which component of the EE was responsible for the increase, it was shown that voluntary exercise on a running wheel by itself increased neurogenesis (van Praag et al. 1999b). Exercise

and enrichment appear to play different roles in this process, with exercise increasing cell proliferation and EE enhancing survival of newborn cells (Olson et al. 2006). When these treatments are done in succession, exercise to increase proliferation followed by EE to promote survival, the effects are additive, with even greater neurogenesis than either treatment alone (Fabel et al. 2009).

Newborn cells in the dentate gyrus contribute to a small, NMDA receptor-independent form of LTP. Thus, increasing neurogenesis through EE or exercise suggests the possibility that plasticity will be altered in the dentate (Snyder et al. 2001; Saxe et al. 2006). It has been shown that this LTP is modestly increased if neurogenesis is increased by preventing the programmed cell death that many newborn cells undergo (Sahay et al. 2011). However, this difference in plasticity might be difficult to detect in typical EE experiments. The increase in neurogenesis caused by blocking cell death ($\sim$100 % increase) is comparable to natural neurogenesis only under optimal conditions where exercise is followed by a period or enrichment, but is much greater than the amount of neurogenesis produced by EE alone ($\sim$35 % increase) (Fabel et al. 2009). Furthermore, this neurogenesis-dependent LTP is masked by a much larger LTP if inhibition is reduced pharmacologically (Snyder et al. 2001), as is often done when testing dentate gyrus LTP in vitro (e.g., Eckert et al. 2010). Still, it is tempting to speculate that EE protocols that also employed a running wheel might exhibit an enhanced plasticity in the dentate gyrus. However, comparing the different studies in Table 1, no such pattern is apparent, possibly suggesting that the effect was too small to detect or that it was masked by structural changes that occur during slice preparation (see section on Methodological Considerations).

2 Memory Improvements

The combined anatomical changes in the hippocampus and neocortex following EE suggest that there may be corresponding enhancements of cognitive function. Indeed, evidence for improved cognitive function in enriched animals had already been demonstrated by Donald Hebb (1949). Hebb investigated the effects of sensory deprivation and enrichment on subsequent cognitive function. In his initial study, he found that rats blinded early in life were cognitively impaired compared to rats blinded as adults. This finding made him wonder whether the converse were true, i.e., whether rats that experienced an enriched sensory environment would show some cognitive benefit. To test this, he took litters of rats home to be raised as pets. When tested later, the pet rats did indeed show signs of cognitive improvement compared to normal laboratory rats.

The cognitive improvement following EE has been demonstrated repeatedly over the years by many researchers, often using hippocampus-dependent memory tasks (Kempermann et al. 1997; Duffy et al. 2001; Teather et al. 2002; Schrijver et al. 2004; Irvine and Abraham 2005; Eckert et al. 2010). As an example of improved memory performance, we recently tested watermaze spatial learning and

Table 1 Summary of electrophysiology findings from EE experiments

EPSP	PS	LTP	LTD	Authors	Species	Sex	Age	Duration (weeks)	Run wheel
				DG in vitro					
NC	NC	NC	?	Eckert et al. (2010)	Rat	Male	P28	12 cont	N
↑	↑	?	?	Green and Greenough (1986)	Rat	Male	P22	4 cont	N
↑	?	↓	?	Foster et al. (1996)	Rat	Male	P40	4 per	N
NC	?	NC	?	Feng et al. (2001)	Mouse	Male	Adult	2 per	Y
				CA1 in vitro					
NC	NC	NC	↓	Eckert et al. (2010)	Rat	Male	P28	12 cont	N
↑	?	NC	?	Foster and Dumas (2001)	Rat	Male	P42	4 per	N
?	?	↑	↑	Artola et al. (2006)	Rat	Male	P50	5 cont	N
↑	↑	↑	?	Malik and Chattarji (2012)	Rat	Male	P25	4 per	N
NC	NC	↑	?	Duffy et al. (2001)	Mouse	Female	P28	8 cont	Y
NC	?	?	?	Parsley et al. (2007)	Mouse	Male	P21	4 cont	Y
?	?	↑	?	Maggi et al. (2011)	Mouse	Female	P21	10 cont	Y
NC	?	NC	?	Bouet et al. (2011)	Mouse	Female	Adult	12 cont	Y
				DG in vivo					
NC	NC	NC	?	Eckert et al. (2010)	Rat	Male	P28	12 cont	N
↑	↑	NC	?	Irvine et al. (2006)	Rat	Male	Adult	3 per	N
↑	↑	?	?	Sharp et al. (1985)	Rat	Male	Adult	3 cont	N
NC	↑	?	?	Sharp et al. (1987)	Rat	Male	Adult	3 cont	N
				CA1 in vivo					
↑/↓	?	?	?	Irvine and Abraham (2005)	Rat	Male	Adult	3 per	N

Age age of EE commencement in postnatal days; *PS* population spike amplitude); *NC* no change; ↑ increase; ↓ decrease; ? not studied; *cont* continuous enrichment; *per* periodic enrichment

memory of rats that experienced EE (with no running wheels), group housing with no enrichment, or isolated housing with no enrichment. The enriched rats showed better spatial learning on a reference memory task than either the socially housed or isolated rats (Eckert et al. 2010). Enriched rats also showed enhanced spatial working memory ability (Irvine and Abraham 2005). These findings are consistent with the breadth of literature showing that enrichment causes neural changes that improve memory function (cited above and reviewed by (van Praag et al. 2000)), and with the related literature that specific manipulations of hippocampal neurogenesis also affect performance on hippocampus-dependent memory tasks (Sahay et al. 2011; Creer et al. 2010; Arruda-Carvalho et al. 2011).

Enriched environment has also been shown to be beneficial for a wide variety of abnormal or pathological brain conditions. Animal models of stroke, epilepsy, and mental retardation all show improvement following EE (Ohlsson and Johansson 1995; Young et al. 1999; Begenisic et al. 2011). Animal models of psychiatric disorders, including anxiety and depression, also respond positively to EE treatment (Cui et al. 2006; Hendriksen et al. 2012). Although controversial, one hypothesis is that a depletion of brain-derived neurotrophic factor (BDNF) leads to the development of depression (Chourbaji et al. 2011). BDNF is a key mediator of plasticity and it has been shown that LTP in the hippocampus is impaired due to reduced BDNF in an animal model of depression (Yeh et al. 2012). Interestingly, antidepressant medications as well as EE/exercise increase BDNF levels, particularly in the hippocampus (Alme et al. 2007; Falkenberg et al. 1992; Farmer et al. 2004). Thus, it is possible that improved hippocampal plasticity contributes to improved mood in depression, although complex psychiatric disorders almost certainly involve altered processing in a number of brain areas outside the hippocampus.

Enriched environment can also ameliorate a number of neurodegenerative disorders, including Parkinson's, Huntington's, and Alzheimer's (for review see Nithianantharajah and Hannan 2006). Alzheimer's disease (AD) pathology is characterized by amyloid plaque deposition and neurofibrillary tangles. In the end stages of the disease, this pathology is widespread throughout the brain, but in the early stages, the medial temporal lobe is particularly affected, with the hippocampus and entorhinal cortex being among the first regions to show amyloid plaque deposition (Braak and Braak 1991, 1995). As a consequence, profound memory impairment is one of the hallmark symptoms that contributes to the progressive cognitive decline. In mouse models of AD, EE treatment can improve memory performance and reduce amyloid deposition in both the hippocampus and cortex (Lazarov et al. 2005; Valero et al. 2011).

3 Methodological Considerations

The anatomical and cognitive changes that occur in EE rats indicate that neural plasticity in the hippocampus is occurring, but there remains the issue of what changes in cellular function and network dynamics mediate the enhanced cognitive

function. A number of studies have attempted to elucidate how the network properties are changed in EE animals, but before describing them, it is worth noting that there is one consistent factor that complicates the study of EE, namely the type of the enrichment procedures used in different laboratories. There are many variables that contribute to environmental enrichment, such as size of the housing, number of animals in the environment, the variety and arrangement of objects, the frequency the objects are changed, etc. The duration of the exposure is also important, including both the total exposure time and whether the exposure is continuous or periodic. A final important factor is the opportunity for exercise, such as that provided by a running wheel. As mentioned above in the case of neurogenesis, exercise can have different and even additive effects on plasticity compared to those of enrichment alone (Fabel et al. 2009). Different laboratories use different combinations of these variables, a fact that complicates interpretation of experimental results.

However, the argument has been made that variation in methods actually helps to improve the reliability and reproducibility of findings from different labs (Wolfer et al. 2004; Richter et al. 2009). A recent study using inbred mouse strains compared standardization versus heterogenization of factors such as animal age and cage enrichment on several behavioral tests (Richter et al. 2011). While they found that heterogenization helped to reduce some of them between lab variability in results, there still remained a large difference between labs. Others have argued that heterogenization should be employed cautiously and that the goals of the experiment must be taken into account (van der Staay et al. 2010). Since EE is the experimental factor being tested in the studies reviewed here, some standardization of EE protocols seems warranted to try and reduce the variability in results between labs.

4 How Does EE Affect Cellular Function?

A number of studies have shown that EE can affect the molecular components of the brain. The early studies demonstrating increased cortical weight and thickness also reported an increase in acetylcholinesterase activity in EE rats (Rosenzweig et al. 1962; Bennett et al. 1964). Levels of brain-derived neurotrophic factor (BDNF), which is involved in plasticity, increase in the hippocampus and cortex following EE (Falkenberg et al. 1992; Farmer et al. 2004). EE can also reduce inhibition in the cortex to promote plasticity (Sale et al. 2007; Baroncelli et al. 2011). Other neuromodulators including serotonin and dopamine are also altered during EE (Simpson and Kelly 2011).These molecular changes suggest that cellular function in the brain is significantly altered during EE, giving rise to changes in network dynamics that support improved cognitive function. In this review, we will focus on how EE affects the physiology of the hippocampus, using the hippocampal memory system as a model for higher cognitive function.

Many studies have tried to find changes in the electrophysiological properties of the hippocampal neurons of EE animals that might explain the improved cognitive function. Two common hypotheses for such experiments are that EE caused a learning-induced change in synaptic strength and/or that EE caused a change in long-term plasticity mechanisms themselves, increasing or decreasing the ability of synapses to undergo further strengthening or weakening. If EE caused a change in synaptic strength, then this should be evident as a difference in basal synaptic transmission, i.e., the size of evoked field potential responses in EE animals should be different than those of control animals, or the frequency or amplitude of miniature EPSCs should change in patch-clamp experiments. Testing for possible changes in plasticity mechanisms typically involves assessment of long-term potentiation (LTP), including the amount of potentiation induced and its persistence over time, although long-term depression (LTD) of synaptic efficacy has also been studied. If EE causes a change in plasticity mechanisms, then this should be evident as either an increase or decrease in the amount of LTP/LTD induced. Predicting either an increase or decrease in LTP in EE animals depends to some extent on whether there is an observed change in basal synaptic transmission. If EE caused an increase in synaptic strength, then this might be expected to occlude the induction of further potentiation, resulting in a reduced LTP in EE animals. Alternatively, if there was no EE-induced change in synaptic transmission, then it might be expected that more LTP would be induced in EE animals.

EE treatment may also alter short-term plasticity. Short-term plasticity is typically assessed with paired-pulse stimulation. In the hippocampus, it is typical for the ratio of the synaptic response to the second pulse to be larger than the response to the first pulse, reflecting an increase in presynaptic transmitter release (paired-pulse facilitation, PPF). In contrast, cell-firing might be reduced in response to the second pulse, as a result of activating GABAergic synaptic inhibition (paired-pulse inhibition). A reduction in the PPF ratio in EE animals is typically interpreted as reflecting an increase in the basal probability of presynaptic transmitter release under EE conditions, while a reduced paired-pulse inhibition of cell firing is interpreted as a reduction of either feed-forward or feed-back GABAergic inhibition.

Testing for EE-induced changes in these measures is typically done with field potential recordings in ex vivo brain slices. Here, animals are differentially housed for a period of time and then brain slices are prepared and measurements made in vitro. The preparation of ex vivo brain slices involves a rapid dissection of the brain, followed by rapid chilling and cutting. The resulting slices are then maintained in a recording chamber that is perfused with oxygen-rich artificial cerebrospinal fluid. This method of recording offers the advantage of precise electrode placement as well as rapid administration and washout of any pharmacological agents used. Accurate electrode placement is critical as it can affect the size of the evoked field potential, possibly adding variability when testing for differences in basal synaptic transmission between EE and control animals Indeed, the in vitro studies we describe next have demonstrated a confusing array of results regarding both changes in basal synaptic transmission and plasticity.

5 In Vitro Evidence for Changes in Cellular Function

Green and Greenough (1986) provided the initial report of EE-induced enhanced basal synaptic transmission, in this case in the dentate gyrus of rats that were exposed to an EE for 4 weeks. They observed increased perforant path-evoked excitatory postsynaptic potentials (fEPSPs), which reflect synaptic efficacy, and population spikes (PSs), which reflect the number of postsynaptic cells that discharge action potentials. These changes were evident if measurements were made immediately after EE, but not if measurements were made several weeks later, suggesting that exposure to the EE only transiently increased the strength of perforant path synapses. A similar enhancement of synaptic transmission in the rat dentate gyrus, as well as in CA3–CA1 synapses, has been reported by others (Foster and Dumas 2001; Foster et al. 1996, 2000). Malik and Chattarji (2012) also reported an increase in basal synaptic transmission in CA1, but this was not accompanied by an increase in mini EPSC amplitude. Rather it was accompanied by an increase in mini EPSC frequency without an apparent change in release probability, suggesting that the increase was due to more synaptic contacts.

The evidence from these studies indicates that EE causes an increase in synaptic transmission in the hippocampus, suggesting either more or stronger synaptic connections, or both. It is important to note, however, that there are also reports that fail to observe changes in synaptic strength following EE, both in the dentate gyrus (Feng et al. 2001) and CA1 (Zhu et al. 2011; Bouet et al. 2011; Parsley et al. 2007; Duffy et al. 2001). As discussed above, enrichment protocols differ between labs so it is possible that the discrepancy between reports might be due to differences in protocols. This problem led us to speculate that a long-term EE protocol would promote the greatest effects on hippocampal synaptic transmission and help to clarify the past discrepancies in the literature. Accordingly, we housed rats in an EE continuously for a minimum of 3 months, much longer than the typical duration of approximately 4–6 weeks. Control animals were either socially housed in standard cages or singly housed. As in the previous studies, at the end of the enrichment period, we prepared ex vivo brain slices and tested for changes in synaptic transmission. To our surprise, we did not observe any differences in synaptic transmission in either the medial or lateral perforant path inputs to the dentate gyrus, or in the CA3–CA1 inputs to stratum radiatum or stratum oriens. We also examined the perforant path responses in vivo but again did not observe any differences between EE and control animals (Eckert et al. 2010).

There are several reasons that may account for the discrepant results. The variable findings might be real and due to differences in EE protocols. On the other hand, it may be that a shorter exposure to EE yields detectable changes, whereas after longer exposures a homeostatic readjustment of synaptic strengths occurs, resulting in no detectable effect at 3 months (Turrigiano 1999, 2008). It may also be that ex vivo brain slices are not well suited to measuring potentially subtle changes of synaptic strength that occur in vivo. Something in the process of generating slices may alter or abolish the network of synaptic weights that existed

in vivo. We will discuss this point in more detail later. However, even if the exact state of synaptic weights is not preserved in slices, it may be reasonable to assume that plasticity mechanisms are intact, as well as any changes to these mechanisms that may have occurred during the enrichment.

Changes to short-term plasticity in EE animals have been examined previously, but most previous *in vitro* studies have not observed a change in paired-pulse facilitation in either CA1 (Parsley et al. 2007; Foster and Dumas 2001; Malik and Chattarji 2012) or the dentate gyrus (Foster et al. 1996; Feng et al. 2001). In agreement with these studies, we did not observe an alteration in PPF in either CA1 or the dentate gyrus following a longer period of EE (Eckert et al. 2010). Past studies have not examined the issue of network inhibitory dynamics using paired-pulse inhibition, but when we examined this issue, we did observe a modest but significant effect of PPI in the stratum oriens of CA1. This was expressed as an interaction effect whereby EE slices exhibited more population spike inhibition that controls at short (<30 ms) intervals, but less inhibition at longer intervals. There was a similar trend observed in the stratum radiatum, but it was not statistically significant. Thus, while EE appears not to alter presynaptic transmitter release, there is a subtle but complex effect on inhibition in CA1, at least following a long period of EE exposure.

Long-term plasticity is typically measured by inducing long-term potentiation and long-term depression. LTP- and LTD-like changes are believed to underlie memory formation (Martin et al. 2000; Martin and Morris 2002) and there is experimental evidence that this is true for hippocampus-dependent memory tasks (Whitlock et al. 2006; Pastalkova et al. 2006). Enrichment might affect LTP/LTD in a number of ways. If learning and LTP share similar mechanisms, and if there were any changes in synaptic strength during the enrichment, then these might occlude the subsequent induction of LTP (Moser et al. 1998; Castro et al. 1989). Alternatively, the plasticity mechanisms may be enhanced during EE such that more LTP/LTD is induced, or is more persistent, in slices from EE animals. It may also be the case that EE does not alter plasticity, at least in a way that can be detected as a change in LTP or LTD. Evidence for all of these outcomes exists in the literature.

In the same study that reported an increase in dentate synaptic transmission in slices from EE animals (Foster et al. 1996), there was also less LTP in the slices from EE animals. The authors suggested that reduced LTP occurred because the synaptic enhancement that occurred during the EE had partially exhausted the mechanism for LTP, thus occluding further LTP induction by electrical stimulation. The occlusion principle assumes that the plasticity mechanism itself is not altered by enrichment, i.e., there is a limited dynamic range in which changes in synaptic strength can occur and this remains constant during enrichment. Alternatively, if the plasticity mechanism is altered by EE, then the amount of LTP can extend beyond the normal range. Artola et al. (2006) observed this effect in the CA1 region of EE animals. They observed an increased LTP as well as greater LTD in slices from EE animals suggesting that EE had increased the dynamic range for synaptic strengths.

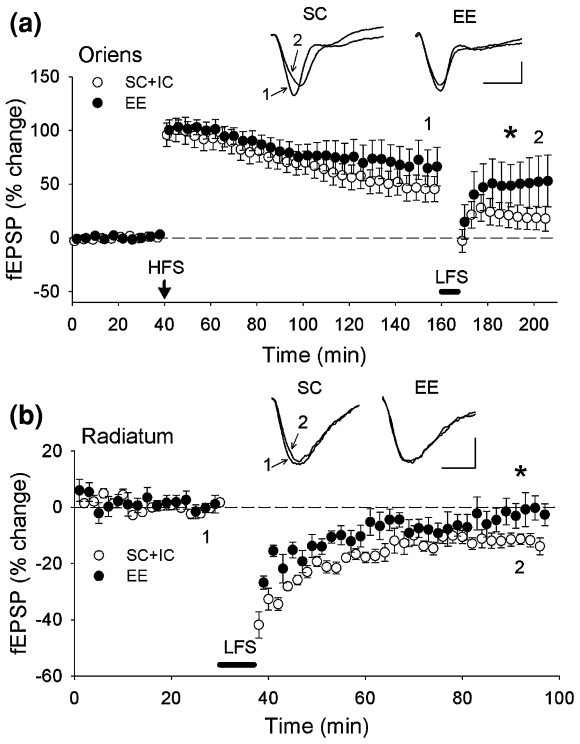

Fig. 1 Ex vivo brain slices reveal subtle changes in plasticity in CA1 following 3 months of EE. **a** In the stratum oriens, LTP induction by high-frequency stimulation (HFS) and persistence is similar in enriched (EE) and socially housed control rats, either individually caged or socially housed (SC + IC). Attempted reversal of LTP by low-frequency stimulation (LFS) revealed a significant depotentiation in SC + IC but not EE rats, indicating a more stable LTP in the EE condition. **b** In a different experiment in the stratum radiatum, EE rats showed a significantly reduced amount of de novo long-term depression following LFS. In both panels, inset average waveforms show representative responses recorded before and after LFS for an EE and SC animal, at the time points indicated. Figure adapted with permission from Eckert et al. 2010

An enhanced plasticity mechanism does not necessarily imply that the dynamic range for synaptic strength will increase, as it could be that the threshold for LTP induction is reduced or that its persistence is changed. Using a relatively mild tetanus, some have observed a more persistent LTP in the CA1 region from EE mice (Maggi et al. 2011; Duffy et al. 2001). One hour after induction, the LTP in control slices had decayed back to baseline levels whereas LTP continued to persist in EE slices. In our study of extended EE treatment, we also obtained evidence for a more persistent LTP in CA1 (Eckert et al. 2010). In the stratum oriens, there was no difference in the amount of initial LTP between enriched and control slices, nor was there a difference in the decay during the 2 h following induction. However, when a low-frequency stimulus was applied to reverse the potentiation, the LTP in EE slices was resistant to reversal and remained elevated whereas the LTP in control slices was significantly reduced (Fig. 1a). We also observed a small but significantly reduced amount of de novo LTD in the stratum radiatum of EE slices (Fig. 1b). Together with the previous findings (Duffy et al.

2001; Maggi et al. 2011), and a recent study demonstrating increased LTP in whole-cell recordings of CA1 neurons (Malik and Chattarji 2012), one is tempted to conclude that EE alters plasticity in the CA1 region of the hippocampus in a way that biases synaptic changes toward strengthening, in the absence of changes in basal synaptic transmission. However, as with the differing reports of EE effects on synaptic transmission, there is no clear consensus on whether EE affects hippocampal plasticity, with reports of EE having no effect on LTP induction and persistence in either the dentate gyrus(Feng et al. 2001) or CA1 (Bouet et al. 2011; Foster and Dumas 2001; Huang et al. 2007). Thus, it is difficult to draw a simple conclusion regarding the effect of EE treatment on synaptic plasticity mechanisms.

6 How Valid are In Vitro Measurements?

The variability in results from these different in vitro studies, as well as our lack of evidence for altered synaptic transmission following a prolonged period of EE, led us to consider the possibility that ex vivo brain slices may not faithfully preserve the in vivo state of synaptic strengths. As in the case of enrichment protocols themselves, the procedures for preparing brain slices differ between labs and thus introduce possible sources of variation in results. Even if the procedures were consistent between labs, the removal of the hippocampus from the brain could affect its network function, especially considering the cooperative interaction between the hippocampus and neocortex posited to occur during memory storage (e.g., McClelland et al. 1995), and during EE (Sutherland et al. 2010). Cutting the hippocampus into slices after its removal offers further obvious opportunity for disrupting network function. One step common to dissection procedures, the rapid chilling of the hippocampus to $\sim$4 °C, has dramatic effects on cell ultrastructure. Kirov et al. (2004) used electron microscopy to show that there is a complete retraction of dendritic spines when a slice is chilled and a subsequent excessive proliferation of spines upon rewarming. Such remarkable changes have the potential at least to mask, if not completely eliminate, any changes in synaptic strength that occurred in vivo.

To test directly whether our ex vivo recordings were missing an in vivo effect, we performed in vivo recordings to record changes in dentate gyrus function during both enrichment and LTP induction and then performed slice recordings from the same animals, recording from the same area of hippocampus where the in vivo measurements were made (Eckert and Abraham 2010). In the first experiment, rats were housed in an EE for 7–15 days, a time period we have previously shown to induce a small but reliable increase in synaptic transmission and a large robust change in excitability (Irvine et al. 2006). During the in vivo recording, we did not on this occasion detect the small change in synaptic transmission, but we did observe a significant increase in excitability as measured by the increase in PS amplitude. When recordings were done in vitro from the same animals, neither the basal fEPSP nor PS was significantly different between EE and control slices,

suggesting a slicing-induced reversal of the excitability increase. However, since the effect of EE was possibly not great enough to appear in ex vivo recordings (which do not benefit from the within-animal recordings before and during EE that the in vivo protocol offers), we switched to inducing LTP in the dentate gyrus in vivo, using a strong tetanus protocol that we have previously shown to induce a large and persistent potentiation (Abraham et al. 2002). The LTP induced in vivo yielded robust changes in both the fEPSP and PS when measured 24 h after induction. Ex vivo brain slices were immediately prepared from these animals, using the non-tetanized hemisphere as a within-animal control for the in vitro measurements. Surprisingly, we still failed to detect a difference in fEPSP size between the tetanized and control slices. However, a greater PS amplitude was detectable in the slices from the tetanized hemisphere compared to the control hemisphere slices. Although the increase was modest compared to the increase observed in vivo, the in vitro and in vivo measurements were significantly correlated. These data led us to conclude that while cell excitability changes can be preserved relatively well in ex vivo slices, LTP induced in vivo may not be reliably reported by in vitro recordings. Variations in the degree of this LTP erasure during slicing procedures may explain some of the differences between studies in the effects of EE on basal levels of synaptic transmission.

7 In Vivo Measures of Cellular Function

A clear alternative to the use of ex vivo brain slices is to perform recordings in vivo. Besides providing a more accurate and online measure of any synaptic changes, they allow measurements to be made across many days, providing a time-course for the effects of enrichment. They do have the disadvantage that there is more variability in electrode placement, which can affect the sizes of the baseline field potentials, but this can be controlled for by normalizing the data prior to the EE treatment.

Using in vivo recordings in area CA1 of freely moving rats, we observed that EE produced a complex effect on synaptic transmission (Irvine and Abraham 2005). Input–output curves measured before and after 3 weeks of overnight EE (during the day, animals were housed in their home cage) revealed a decrease in the size of the fEPSP at low stimulation intensities and an increase at high stimulation intensities. This result suggests that EE produces an effect more complex than a single process of synaptic enhancement, and perhaps involves alterations to neuromodulatory systems. The net effect of this change in the input–output curve would be an increase in the dynamic range of synaptic strengths such that a given range of inputs are spread out among a larger range of outputs in EE animals. An expanded range of synaptic strengths implies a greater capacity for information storage in the hippocampus, and as it has recently been shown that the excitability of CA1 neurons increases following EE (Malik and Chattarji 2012), a potential increase in the information propagated back to the neocortex. Although these

hypotheses need to be tested further, they do suggest a beneficial change in the network dynamics such that information is processed and stored more efficiently in CA1 of EE animals.

In the dentate gyrus, 3 weeks of overnight EE produced a moderate but significant increase in the fEPSP in vivo (Irvine et al. 2006). Interestingly, this effect depended on the nature of the rats' home cages in which they were housed during the day. If the home cages were hanging wire mesh cages, then there was no effect of overnight EE evident in fEPSP measurements. It was only when the home cages were plastic and lined with bedding material that the effect was apparent (Fig. 2a). Although not known for certain, we attributed this difference to a possible effect of stress from the wire mesh cages. Regardless of the exact cause, the lack of observed change in the wire mesh home cage group, as well as the modest ($\sim$10 %) increase in the fEPSP in the plastic home cage group, suggest that the synaptic effects of EE are subtle and subject to possible disruption by other environmental factors that the animal experiences.

Enrichment had a much more pronounced effect on excitability in the dentate gyrus, an effect that was evident in both the wire mesh and plastic home cage groups (Irvine et al. 2006) and has been observed previously (Sharp et al. 1985, 1987). After starting the overnight EE, the population spike amplitude increased significantly and on average was doubled after 10 days of EE (Fig. 2b). The PS amplitude then began to decrease, even though EE treatment continued, so that at the end of the 3 weeks of EE the PS increase had decayed by 50 %. Upon cessation of enrichment, the PS continued to decay back to original baseline levels over the next 10 days. The magnitude of the PS change was comparable in both wire mesh and plastic home cage groups suggesting it was a more robust change than the synaptic effect and less susceptible to the influence of other environmental factors. Furthermore, the excitability change showed a 'dose-dependent' effect of enrichment, as an additional group of animals that experienced only 1 h/day of EE showed only a 50 % increase in PS amplitude compared to the 100 % increase observed in the overnight EE group.

When LTP induction was tested in these animals, there was no effect of EE on the amount of LTP induced as both controls and EE groups showed similar increases in fEPSP slope. This suggests that the EE-induced EPSP change was either too small to occlude subsequent LTP induction, or that there was a metaplastic effect that masked any occlusion. Despite the lack of difference on LTP induction, there was a significant difference in persistence between EE and control animals. LTP in EE animals decayed faster than in control animals, returning to baseline levels within 3 days, whereas LTP decayed slowly in control animals and was still significantly above baseline levels 2 weeks later. This remarkable effect could be due to the fact that LTP induction occurred while EE was ongoing and thus the reversal may have occurred because the potentiation was more labile and not fully consolidated. However, we have observed a similar EE-induced reversal of a more stable LTP (Abraham et al. 2002). In this experiment, LTP induction in the dentate gyrus resulted either in a decremental form of LTP that decayed back to baseline levels within $\sim$1 week, or a stable form of LTP that persisted for months, and even up to a

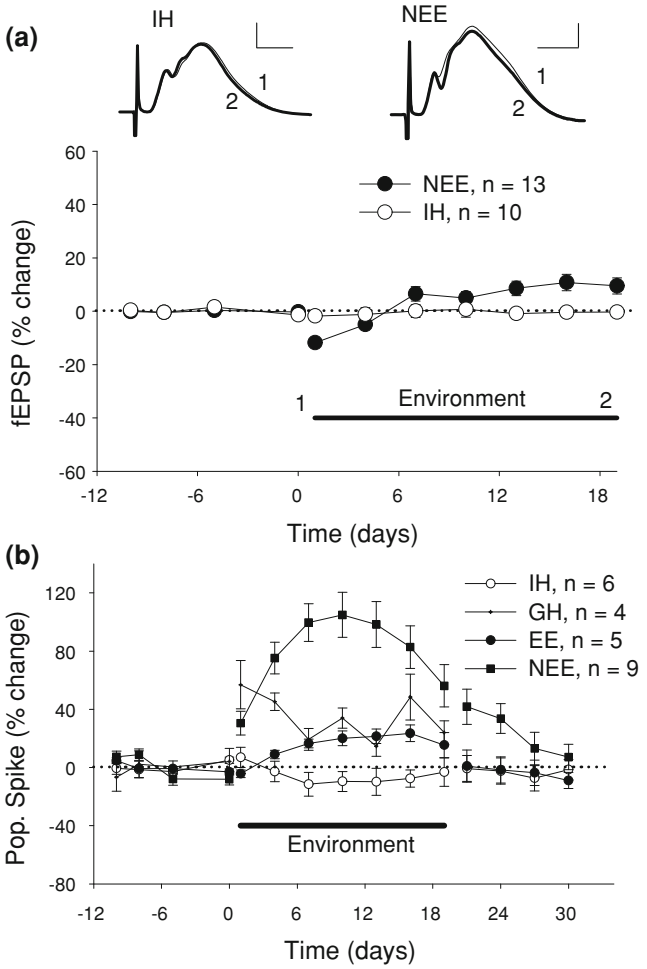

Fig. 2 In vivo recordings during 3 weeks of EE showing changes in synaptic transmission and excitability in the dentate gyrus. **a** Overnight enrichment (NEE) caused a small but significant increase in synaptic transmission compared with singly housed controls (IH). **b** The same EE treatment caused a large but transient change in excitability. In NEE rats, the population spike amplitude doubled after 10 days and then began to decline thereafter even though EE continued. The effect on excitability was dose-dependent as a lesser EE treatment (1 h/day; EE) and a shift from single to group housing (GH) produced smaller effects. Inset average waveforms show representative examples from IH and NEE rats before and at the end of enrichment. Figure adapted with permission from Irvine et al. 2006

year. When a group of animals that exhibited stable LTP was subjected to a mild EE treatment of 1 h/day, the LTP was partially reversed back to baseline levels (Fig. 3a). When the dose of EE was increased to overnight exposure in another group of animals the LTP was completely reversed (Fig. 3b). Interestingly, the LTP reversal was evident when the EE treatment began 14 days after LTP induction but

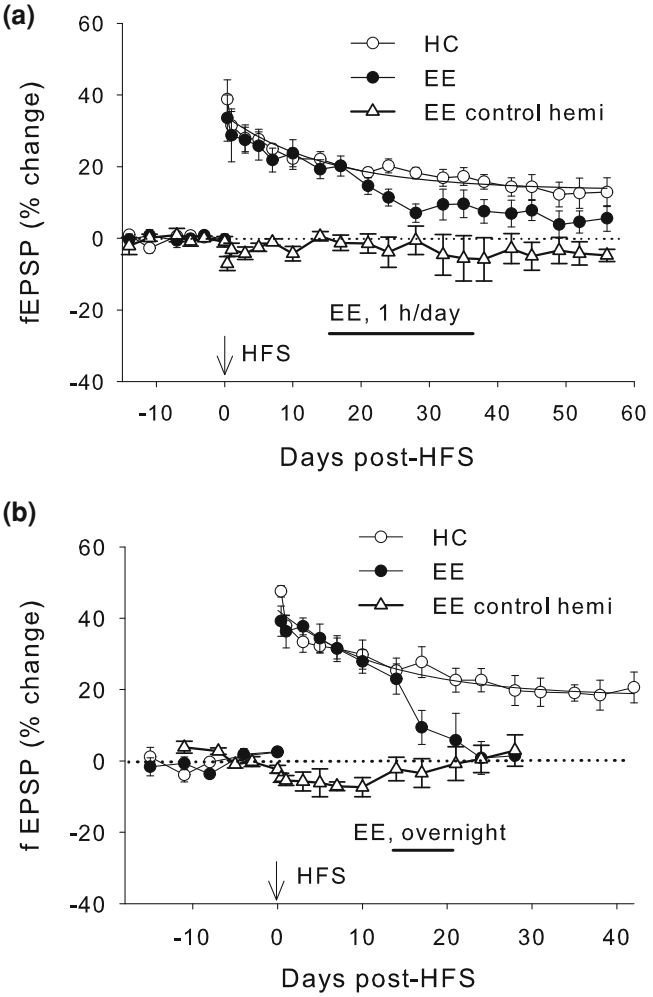

Fig. 3 EE reverses stable LTP in the dentate gyrus in a dose-dependent manner. **a** Two weeks after the induction of LTP, a small dose of EE (1 h/day for 2 weeks) caused a partial reversal of LTP (EE group). Singly housed home cage control animals (HC) exhibited a relatively stable potentiation. An exponential curve fit in control animals had an asymptote of 13 % LTP. The non-tetanized control pathway in the contralateral hemisphere (EE control hemi) remained stable throughout LTP and EE treatments **b** Increasing the EE treatment to overnight for 7 days caused a complete reversal of LTP (EE). Controls (HC) again exhibited a stable LTP with an asymptote of 18 %. Figure adapted with permission from Abraham et al. 2002

not when it commenced 70 days after indicating an eventual consolidation of LTP across time, making it resistant to EE-induced reversal. Like LTP, LTD can also be reversed by EE exposure (Abraham et al. 2006).

The reversal of LTP in EE animals may reflect a disruption of the maintenance mechanism of LTP and a consequent loss of stored information. Alternatively, the

increased cognitive demand of the enrichment may require a more dynamic form of plasticity in which case the reversal of LTP may reflect an enhanced information processing capability of the hippocampus in EE animals. The persistent LTP observed in regularly housed rats (Abraham et al. 2002) may be because the relatively limited stimulus conditions place little demand on the hippocampus. Under these conditions, there may be very little plasticity induced by the environment, allowing for any experimentally-induced plasticity to persist. Support for this idea is given by artificial neural network (ANN) simulations (Abraham and Robins 2005). ANNs provide the opportunity to monitor connection strengths between units as the network stores information. In order for an ANN to store information, connection strengths between units must change. If the network has previously stored information and tries to store new information, the connection weight changes induced by the new learning can cause catastrophic forgetting of the previously learned information. This can be avoided if the old information is interleaved (rehearsed) with the new information. In this situation, there is a redistribution of weights such that both the old and the new information are represented. Thus, the reversal of LTP by EE might reflect this process of ongoing plasticity that is necessary to incorporate new information into the hippocampal network.

It is worth noting that, as in the case of neurogenesis mentioned above, there appears to be a difference between enrichment and exercise on hippocampal synaptic function. While the data from in vitro studies is inconclusive, our studies in vivo suggest that 3 weeks of EE causes subtle changes in synaptic transmission in both CA1 and the dentate gyrus, as well a robust increase in cell excitability in the dentate (Irvine and Abraham 2005; Irvine et al. 2006). A similar effect has been reported in CA1 (Malik and Chattarji 2012). In the case of exercise, however, there appears to be no effect on basal synaptic transmission or excitability (Reisi et al. 2008; van Praag et al. 1999a). The effect of EE on LTP induction is again inconclusive from in vitro studies, but our in vivo data suggest that LTP induction is not altered by EE that does not include running wheels (Irvine et al. 2006; Eckert et al. 2010), although it does affect its persistence (Abraham et al. 2002, 2006; Irvine et al. 2006). Contrary to this, exercise does facilitate LTP induction (van Praag et al. 1999a; Farmer et al. 2004). Thus, the different effects of enrichment and exercise on neurogenesis and synaptic function suggest that the spatial and sensory complexity of an EE alters the hippocampal network differently compared to a period of exercise alone.

8 Evolution of Enrichment Effects on Hippocampal Function

The evidence for EE-induced changes in hippocampal physiology, although somewhat varied, suggests a possible sequence of changes that occur, with early changes in synaptic transmission and excitability gradually giving way to more subtle changes in network dynamics.

The robust increase in dentate gyrus cell excitability following EE (Irvine et al. 2006) represents a significant increase in activity levels in the dentate and may contribute to the downstream changes in CA1. The doubling of the granule cell PS amplitude would approximately double the drive onto CA3 neurons as well as indirectly increase activity in CA1. To our knowledge, the effect of EE on CA3 function has not been examined, but it is possible that the increased activity facilitated some of the changes that have been reported in CA1, including the complex change in input–output curves observed in vivo (Irvine and Abraham 2005), and the increase in excitability and LTP observed in vitro (Malik and Chattarji 2012; Duffy et al. 2001).

Apart from affecting network activity downstream from the region in question, the increased excitability would also be expected to promote a period of heightened local synaptic plasticity. A period of heightened plasticity following a short duration of EE could account for the observed changes in plasticity observed in vitro, including an increase in LTP (Malik and Chattarji 2012) and an increase in dynamic range of synaptic efficacy (Artola et al. 2006). It may also explain why LTP induced in vivo is readily reversed by enrichment (Abraham et al. 2002, 2006).

It is notable that the increase in dentate excitability was transient, peaking around 10 days after the start of the EE treatment and declining thereafter. Indeed, in animals that were housed continuously in an enriched environment for 3 months, there was no difference from controls in either EPSP or PS measures (Eckert et al. 2010). It is possible that some of the changes in CA1 are transient as well. The in vitro studies suggest that this is at least partially true, as the increases in synaptic transmission, excitability, and plasticity observed following short periods of EE (Foster and Dumas 2001; Malik and Chattarji 2012) were not observed, with the exception of subtle changes in plasticity and recurrent inhibition, following a longer period of EE (Eckert and Abraham 2010). It is possible that the whole hippocampus undergoes a transient period of increased activity and plasticity during the initial exposure to EE, but then returns to pre-EE levels in the long-term, possibly due to homeostatic adjustments (Turrigiano 1999, 2008). Thus, the changes in cellular physiology of the hippocampus that support improved cognition in the long-term may not be large, persistent changes in synaptic strength, or striking differences in plasticity. It may be instead that there are long-lasting but subtle changes in network dynamics or plasticity capability that are not readily detectable with field potential recordings. Other techniques, such as recordings of place cell activity in freely moving animals may provide interesting insights into the network dynamics of the hippocampal formation.

Acknowledgments The work presented from the Abraham laboratory was supported in part by grants from the Royal Society of New Zealand Marsden fund and a James Cook Fellowship to W.C.A.

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All About Running: Synaptic Plasticity, Growth Factors and Adult Hippocampal Neurogenesis

Carmen Vivar, Michelle C. Potter and Henriette van Praag

Abstract Accumulating evidence from animal and human research shows exercise benefits learning and memory, which may reduce the risk of neurodegenerative diseases, and could delay age-related cognitive decline. Exercise-induced improvements in learning and memory are correlated with enhanced adult hippocampal neurogenesis and increased activity-dependent synaptic plasticity. In this present chapter we will highlight the effects of physical activity on cognition in rodents, as well as on dentate gyrus (DG) neurogenesis, synaptic plasticity, spine density, neurotransmission and growth factors, in particular brain-derived nerve growth factor (BDNF).

Keywords Adult neurogenesis · Dentate gyrus · Running · Learning and memory · Neurotrophic factors · Synaptic plasticity · Endurance factors

Contents

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Curr Topics Behav Neurosci (2013) 15: 189–210
DOI: 10.1007/7854_2012_220

Published Online: 31 July 2012

1 Exercise and Cognition

Cognition studies in adult rodents have shown that both voluntary and forced exercise enhance spatial memory in the Morris water maze, Y-maze, T-maze and radial arm maze tests (Fordyce and Farrar 1991; van Praag 2008). Running also improves performance on tasks that require minimal spatial navigation. For example, contextual fear conditioning, passive avoidance learning and novel object recognition are enhanced by running (Baruch et al. 2004; Falls et al. 2010; Liu et al. 2008; Mello et al. 2009; O'Callaghan et al. O'Callaghan et al. 2007; Hopkins and Bucci 2010; Fahey et al. 2008; Griffin et al. 2009). It remains to be determined whether the beneficial effects of exercise for performance on these tasks is mediated by the hippocampus and/or whether other brain areas are involved to a similar extent. For example, contextual fear conditioning depends on both the hippocampus and amygdala (Phillips and LeDoux 1992; Myers and Gluck 1994; Wiltgen et al. 2006; Maren 2001, 2008). The hippocampus is critical for memory of context; whereas the amygdala stores the context–shock association (Huff and Ruddy 2004). However, the effects of physical activity on the amygdala are still unclear. Greenwood et al. (2009) showed that 6 weeks of running before conditioning improves hippocampus dependent memory for context, but not extinction (amygdala-dependent), suggesting that voluntary physical activity selectively increased hippocampus-dependent memory. Subsequent studies showed that physical activity improved learning and consolidation of cued conditioned fear, an amygdala-dependent process, but not the retrieval or performance of conditioned fear (Falls et al. 2010; Lin et al. 2012). Interestingly, exercise increases BDNF mRNA, and synaptic proteins such as TrkB and SNAP-25 levels in both the dentate gyrus (DG) and amygdala (Greenwood et al. 2009; Lin et al. 2012), albeit more so in the DG (Greenwood et al. 2009). Thus, physical activity may differentially affect amygdala and hippocampus-dependent plasticity.

Within the hippocampal subfields (area CA1, area CA3, DG), physical activity likely has differential functional effects. Indeed, it has become increasingly well-established that the subfields of the hippocampus may mediate different aspects of memory formation. Area CA1 is deemed important for encoding, area CA3 for pattern completion and the DG is considered to mediate pattern separation (McHugh et al. 2007; Leutgeb et al. 2007; Leutgeb and Leutgeb 2007; Gilbert et al. 2001; Bakker et al. 2008; Gold and Kesner 2005; Kesner 2007; Schmidt et al. 2012). The DG is one of the brain regions with substantial addition of new neurons throughout the lifetime of mammals, (Altman and Das 1965) and it has been suggested that these new neurons contribute significantly to pattern separation. Ablation of adult neurogenesis results in deficits in fine pattern discrimination in the touchscreen (Clelland et al. 2009) and the ability to distinguish between two similar contexts in fear conditioning (Tronel et al. 2010). Spatial pattern separation was evaluated recently in running versus sedentary mice (Creer et al. 2010). Specifically, mice were tested on a spatial discrimination task, where stimuli were presented in close or distal proximity using a touchscreen method that requires

minimal motor activity (Morton et al. 2006). There was no difference between the groups when the separation between stimuli was large, however, runners outperformed sedentary mice when the difference between stimuli was small (Creer et al. 2010). The observed improvement in making fine spatial distinctions may be due at least in part to the increase in adult neurogenesis that is observed in the hippocampus with exercise (van Praag et al. 1999a). Indeed, in a transgenic mouse with increased adult hippocampal neurogenesis there is improved differentiation between overlapping contextual representations, indicative of enhanced pattern separation (Sahay et al. 2011).

2 Neurogenesis and Exercise

Many different extrinsic and intrinsic factors can regulate the production of new neurons. Housing mice in an enriched environment (EE) resulted in the first evidence that adult neurogenesis could be enhanced (Kempermann et al. 1997, 1998). Increased neurogenesis was correlated with improved performance in a hippocampus-dependent spatial learning task, the Morris water maze (Kempermann et al. 1997). However, an enriched environment is a complex combination of inanimate and social stimulation, learning and physical activity. Separation of the different elements of the EE showed that running per se increases cell proliferation, neurogenesis, and synaptic plasticity, as well as spatial memory function in the mouse DG (van Praag et al. 1999a, b; Kronenberg et al. 2003; van der Borght et al. 2007). A potential confound in the initial study was that the positive control, the EE also contained a running wheel (van Praag et al. 1999a). However, at that time the finding that running increased neurogenesis was completely unexpected and so an EE group without a wheel was not included. Subsequent research suggested that running enhanced the number of bromodeoxyuridine (BrdU) positive cells more so than EE without running wheels, but that EE still differed from standard housing conditions (Ehninger and Kempermann 2003), even though only marginally so (Fabel et al. 2009). However, in these studies the control mouse cages were typically smaller than those of the EE group, allowing less activity.

In a recent study, we aimed to dissociate the effects of physical activity and enrichment in young female C57Bl/6 mice housed in identically sized cages under control, running, and EE with or without running wheels. Cell proliferation, neuron survival, and neurotrophin levels were enhanced only when running wheels were accessible, suggesting that exercise is the critical factor mediating increased BDNF levels and adult hippocampal neurogenesis (Kobilo et al. 2011a) ; Fig. 1). This study has been replicated and extended in individually housed male C57Bl/6 mice. Specifically, the authors added more enrichment in the form of dietary treats and continuous addition of novel objects. Even so, only running and running plus enrichment increased neurogenesis. The greatest amount of physical activity was observed in the running only condition and these mice exhibited improved spatial learning in the Morris water maze (Mustroph et al. 2012). Altogether, these

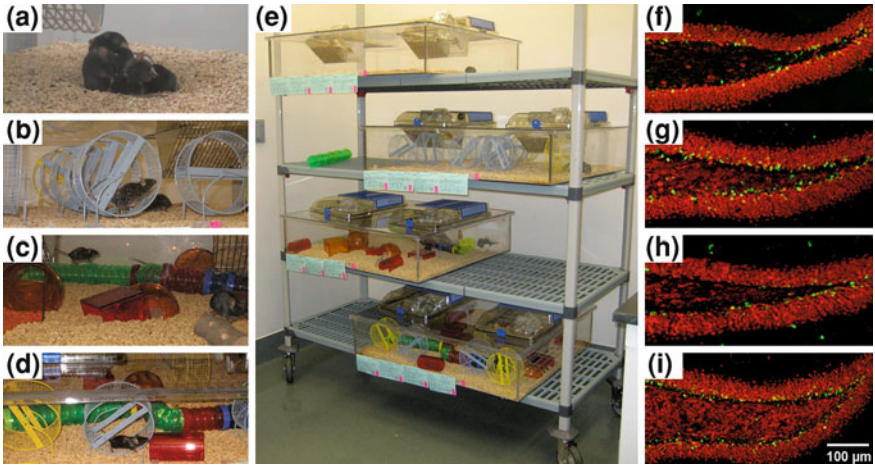

Fig. 1 Exercise but not enrichment increases DG neurogenesis. Female C57Bl/6 mice ($n = 10$ per group) were housed in large cages (30"x33"x8") as follows: **a** Control (CON). **b** Running (RUN). **c** Enriched environment only (EEO). **d** Enrichment and running (EER), this cage contained enrichment objects similar to (**c**), as well as 10 running wheels. **e** Overview of the experimental cages. (**f**–**g**) Confocal images of BrdU-positive cells in the dentate gyrus in sections derived from mice housed in (**f**) CON, (**g**) RUN, (**h**) EEO, or (**i**) EER conditions. Sections were immunofluorescent double-labeled for BrdU (*green*) and NeuN (*red*) indicating neuronal phenotype (Kobilo et al. 2011a)

findings do not preclude the effects of EE only on behavior. Indeed, EE only has effects on synaptic plasticity, such as increased dendritic complexity of young granule neurons (Beauquis et al. 2010), enhanced arborization of cortical neurons (Ip et al. 2002), and elevated synaptophysin levels in hippocampus and cortex (Lambert et al. 2005). Moreover, EE only has been shown to have profound effects on dendritic spines and synapses in cortex and cerebellum (Greenough et al. 1978, 1985, 1986). Therefore, these findings suggest that EE and physical activity share common features pertaining to synaptic plasticity (van Praag et al. 2000), but that the increment in adult neurogenesis and BDNF levels in the hippocampus is specific to physical activity.

Reduced neurogenesis occurs naturally with aging and is observed in certain mouse models of neurodegenerative disease. Exercise may ameliorate or reverse this change in some but likely not all conditions. Indeed, it has been shown that neurogenesis declines as early as middle-age (Kuhn et al. 1996), and may contribute to age-related reductions in cognitive function (Erickson and Barnes 2003). However, the robust effect of exercise on neurogenesis is maintained throughout life in rodents. It has been shown that exercise from 3 to 9 months of age significantly reduced the age-dependent decline in cell proliferation and led to a consecutive increase in the number of more mature cells (Kronenberg et al. 2006). Moreover, in mice that started wheel running in middle age (Wu et al. 2008; Marlatt et al. 2012) or old age (van Praag et al. 2005), new neuron number was

elevated. Furthermore, recent studies have shown that physical activity can reverse radiation-treatment-related decline in hippocampal neurogenesis (Naylor et al. 2008; Wong-Goodrich et al. 2010). In addition, running can ameliorate the genetically reduced generation of proliferating hippocampal cells and enhance the dendritic arborization of newly generated neurons in synRas mice (Lafenetre et al. 2010).

The effects of exercise have also been evaluated in mouse models of neurodegenerative disease. In certain mouse models of Alzheimer's disease (AD), exercise is able to reduce pathology and enhance cognition and adult neurogenesis. In TgCRND8 mice (which express APP695swe, Ind), running improved spatial memory and reduced extracellular Aβ plaque load (Adlard et al. 2005). Exercise was also found to be beneficial in Tg2576 mice (which express APP695swe), even after the onset of pathology (Nichol et al. 2007). Similarly, APOE4 mice have been shown to benefit from regular physical activity (Nichol et al. 2009). Furthermore, in the 3xTG model of the disease, physical activity reduced disease symptoms and improved synaptic plasticity in a recent study (Garcia-Mesa et al. 2011). Reduced neurogenesis in this model (Rodriguez et al. 2008) was improved by running (Rodriguez et al. 2011). Altogether, the effects of exercise appears to be promising in mouse models of AD and is consistent with positive data emerging from studies in humans (Buchman et al. 2012; Lautenschlager et al. 2012).

A notable exception to the beneficial effects of exercise on brain and behavior appears to be in mouse models of Huntington's disease (HD). While initial research showed that EE delays symptom onset in R6/1 transgenic mice (van Dellen et al. 2000), physical activity had equivocal results. In R6/1 mice, running normalized rearing behavior and delayed the onset of deficits in rear-paw clasping, motor coordination and spatial working memory. However, rotarod performance, ubiquitinated protein aggregates, hippocampal BDNF protein levels (Pang et al. 2006; van Dellen et al. 2008), and hippocampal neurogenesis in R6/2 mice (Kohl et al. 2007) were unchanged by exercise. In a recent study, running started in presymptomatic 6-week-old male HD (N171-82Q) mice did not improve function and appeared to accelerate disease onset (shaking, hunched back and poor grooming), reduced striatal volume, and impaired motor behavior compared to sedentary controls. Furthermore, weight loss, reduced lifespan, hyperglycemia, Morris water maze learning deficits, diminished hippocampal neurogenesis, deficits in immature neuronal morphology, intranuclear inclusions, and decreased DG volume were refractory to physical activity (Potter et al. 2010; Fig. 2). It remains to be determined whether similar observations will be made in other mouse models of the disease. Interestingly, a case study in humans also has indicated that physical exercise may not prevent or delay disease onset or progression. In humans, the number of polyglutamine repeats can predict disease onset to some extent (Langbehn et al. 2004). A marathon runner presented with myopathy 20 years before the predicted disease onset for 41 CAG repeats (Altschuler 2006; Kosinski et al. 2007). Thus, the neurogenic and cognitive effects of physical activity should be evaluated carefully across the spectrum of neurological diseases.

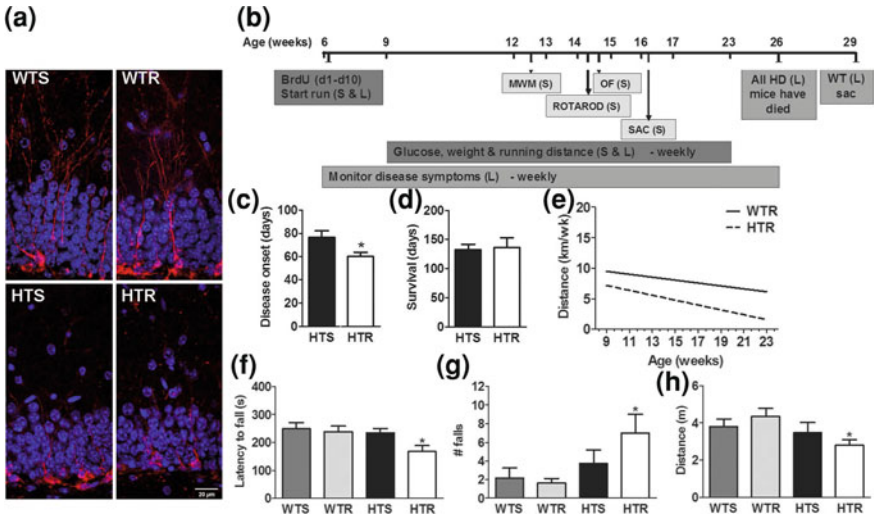

Fig. 2 Exercise is not beneficial in a mouse model of Huntington's disease (HD) N171-82Q. **a** Morphology of Doublecortin (DCX) expressing immature neurons in the DG of WT and HD mice. Dendritic branching complexity of DCX-labeled (*red*) immature neurons was reduced in the transgenic mice, HD sedentary (HTS) and HD runner (HTR) compared to Wildtype sedentary (WTS) and Wildtype runner (WTR). Granular cell layer neurons were labeled with DAPI (*blue*). **b** Timeline of experiment in weeks of age for the subset of mice tested for behavior (S) and mice evaluated over their lifespan (L) mice. BrdU was injected over the first 10 days of the study. Behavioral testing was carried out from week 12 to 15 and mice (S) were sacrificed (SAC) at approximately 16 weeks of age. Survival analysis indicated that HD mice died between 11 and 26 weeks of age. **c** Onset of disease symptoms such as hunched back, poor grooming and involuntary shaking occurred earlier in HD runners; * $p < 0.03$. **d** Lifespan did not change as a result of exercise. **e** There was no significant difference between the groups in running distance over the duration of the experiment. (**f**–**g**) Exercise may exacerbate locomotor deficits in HD mice. (**f**) The latency to fall off an accelerating rotarod was shorter in HTR mice than in all other groups; * $p < 0.03$. **g** The total number of falls from the rotarod over 5 min was increased in HD runners compared to WT mice; * $p < 0.02$, HTR versus WTS and WTR. **h** The total distance traveled over 30 min in an open field was reduced in HD mice compared to WT mice, specifically in HTR mice compared to WTR mice; * $p < 0.03$. Abbreviations: *MWM* Morris water maze; *OF* Open field; *S* Subset tested for behavior; *L* Lifespan group; *SAC* sacrificed (Potter et al. 2010)

3 Synaptic Plasticity: Effects of Exercise On Long-Term Potentiation/Depression, Dendritic/Spine Size and Morphology

Running can influence neural plasticity on many levels, including modifications in synaptic function. Induction of long-term potentiation (LTP), a physiological model of certain forms of learning and memory (Bliss and Collingridge 1993), was measured in hippocampal slices from running and control mice. Field recordings showed a significantly greater LTP in the DG of running mice as compared to controls (van Praag et al. 1999b; Vasuta et al. 2007). Recordings from CA1 region

in the same mice showed no differences between groups, suggesting that the changes observed in the DG were the direct result of increased neurogenesis (van Praag et al. 1999b). Subsequent studies have shown similarly enhanced LTP in vivo in anesthetized rats subject to voluntary (Farmer et al. 2004) and forced running (O'Callaghan et al. 2007). Field recordings in vivo showed a significantly enhanced LTP in the DG induced with a weak theta patterned stimulation which did not produce LTP in control subjects, as well as more short-term potentiation in running rats as compared to controls (Farmer et al. 2004).

Thus exercise modifies the synaptic plasticity of the DG likely as a result of the enhanced neurogenesis. Indeed, recordings of individual newborn dentate granule cells in hippocampal slices have shown that they display a low threshold for LTP induction and enhanced LTP compared to mature granule cells (Wang et al. 2000; Schmidt-Hieber et al. 2004; Ge et al. 2007). This enhanced plasticity was observed in a specific time window (1–1.5 months old) of their maturation process and was dependent on transiently increased synaptic expression of NR2B containing N-methyl-D-asparate (NMDA) receptors (Ge et al. 2007). Together, these results support the hypothesis that newborn granule cells have a unique role in synaptic plasticity of the hippocampus and that their contribution can be enhanced with exercise. Indeed, protective effects induced by exercise on hippocampal plasticity have been observed in middle age to old age rodents. The age-related impairment in expression of LTP was reversed in middle age rodents exposed to regular exercise (O'Callaghan et al. 2009), which correlates with improved memory, enhanced hippocampal neurogenesis and increments of BDNF levels in middle-age rodents exposed to long periods of running (O'Callaghan et al. 2009; Marlatt et al. 2012). It should also be noted that in contrast to the enhanced LTP observed in running rodents, long-term depression (LTD), another type of synaptic plasticity (Bear and Abraham 1996), induced by low frequency stimulation was relatively unaffected by exercise. However, the involvement of NR2A containing NMDA receptors was increased by exercise compared to non-runner mice (Vasuta et al. 2007) suggesting that exercise can alter the contribution of NMDA subunits to LTD.

Modifications in synaptic plasticity have also been associated with morphological changes in response to neural activity (Nägerl et al. 2004). In particular, morphological changes in DG have been observed with exercise (Eadie et al. 2005; Redila and Christie 2006; Zhao et al. 2006; Stranahan et al. 2007). Analysis of individual granule cells revealed that exercise significantly increased the total length, complexity, and spine density of granule cell dendrites (Eadie et al. 2005). Upon classification of individual DG cells by their position in the layer, it was shown that exercise enhances dendritic complexity in all the zones of the granule cell layer (subgranular, inner and outer granule cell zones) (Redila and Christie 2006). Moreover, long-term exercise (2 months) induced morphological changes not only in the DG, but also in the entorhinal cortex and CA1 pyramidal cells (Stranahan et al. 2007).

Use of retrovirus-mediated labeling of newborn neurons has made it possible to examine and characterize morphological details of these cells throughout their lifetime (van Praag et al. 2002). Retroviral labeling showed that exercise does not

modify spine density of newborn granule cells in young compared to aged runner mice (van Praag et al. 2005). A subsequent detailed study in young mice showed that running increased the motility of the dendritic spines of newborn neurons (21 days postvirus-injection) and accelerated their maturation, as quantified by the presence of mushroom spines (28–52 days postvirus-injection), without modifying dendritic complexity (Zhao et al. 2006). Thus, exercise modifies the morphology of dentate granule cells and other parameters related to memory function, and may also influence the rate of integration of newborn granule cells into the hippocampal circuitry.

4 Exercise and Neurotrophic Factors (BDNF, FGF-2, NGF, VEGF and IGF)

Several cellular and molecular systems important for maintaining neuronal function and plasticity, such as neurotrophins may be instrumental for positive effects of exercise on the brain. In particular, brain-derived neurotrophic factor (BDNF) is well known to play an important role in the adult brain in synaptic plasticity (Kuipers and Bramham 2006), learning (Yamada and Nabeshima 2003), and neurogenesis (van Praag 2008; Bekinschtein et al. 2011) and is considered to be the most important factor upregulated by physical activity (Cotman et al. 2007). The first study showing exercise-induced increases in neurotrophins in the hippocampus was by Neeper and colleagues (Neeper et al. 1995). Specifically, they described that 2–7 days of running increases the level of BDNF mRNA in specific brain areas, including the hippocampus. Indeed, BDNF gene and protein expression in the hippocampus were elevated as long as the animals were housed with the running wheels (Berchtold et al. 2005). Furthermore BDNF levels remained upregulated for 2 weeks after running wheels were no longer accessible (Berchtold et al. 2010). Many studies have now demonstrated exercise-dependent changes in BDNF expression (see Cotman and Berchtold 2002, Cotman et al. 2007; Vaynman and Gomez-Pinilla 2005).

A detailed analysis of the hippocampal subfields showed that exercise increases BDNF mRNA levels in the DG but not in CA1 (Farmer et al. 2004). Moreover, exercise induced an increment in the DG of synapsin I, a vesicle-associated phosphoprotein that modulates transmitter release as well as the formation and maintenance of presynaptic structures, suggesting that exercise may induce plasticity in a specific hippocampal subfield, the DG (Vaynman et al. 2004a, b). Changes in the levels of BDNF mRNA and protein, NMDA receptor subunit NR2B mRNA, GluR5 and synapsin I, (Farmer et al. 2004; Vaynman et al. 2004a, b; O'Callaghan et al. 2007) may be associated with the enhanced LTP and neurogenesis observed in the DG of running mice (van Praag et al. 1999b; Farmer et al. 2004; O'Callaghan et al. 2007). Indeed, BDNF is proposed to regulate the synaptic transmission and activity-dependent synaptic plasticity by pre- and

postsynaptic mechanisms (Poo 2001; Binder and Scharfman 2004; Kuipers and Bramham 2006). Genetic deletion of BDNF in mice showed disruption of normal induction of LTP (Korte et al. 1995), a defect that was rescued with BDNF replacement, either through the BDNF-expressing adenovirus (Korte et al. 1996) or by supplying exogenous BDNF (Patterson et al. 1996). Moreover, blockade of tyrosine receptor kinase B (TrkB), the high-affinity receptor for BDNF, with a specific immune-adhesin chimera (TrkB-IgG; Vaynman et al. 2004b) or a TrkB inhibitor (K252a; Liu et al. 2008) was sufficient to inhibit BDNF actions and its beneficial effects on learning and memory.

Over the past years, the effects of exercise on adult neurogenesis and BDNF levels have been extensively studied (for review see van Praag 2008, 2009). Selective ablation of the gene encoding TrkB in hippocampal neural progenitor cells has been shown to prevent the exercise-induced increase in neurogenesis (Li et al. 2008) and the neurogenesis-dependent LTP (Bergami et al. 2008). Using BDNF knockdown by RNA interference using lentiviral vectors injected into the DG has also resulted in reduced neurogenesis (Taliaz et al. 2010). Furthermore, intracerebral infusion of BDNF increases neurogenesis in the DG (Scharfman et al. 2005) and mimics exercise-induced changes in learning (Griffin et al. 2009). The effects of exercise on hippocampal neurogenesis, and BDNF levels have been suggested to be mediated by activation of NMDA receptor containing $\varepsilon 1$ subunit (Kitamura et al. 2003). BDNF's neurogenic effects are area-specific, since BDNF infusion does not induce neurogenic changes in the subventricular zone (Galvão et al. 2008), consistent with data showing that the neurogenic effect of exercise is limited to the hippocampus (Brown et al. 2003).

The beneficial effects of exercise be may to some extent age-dependent. In a recent study, Titterness and colleagues (Titterness et al. 2011) have shown that 13 days of exercise did not increase BDNF protein levels in the DG of adolescent female and male mice (postnatal 22), which correlated with no differences in LTP induction between sedentary and runner female mice. Interestingly, enhanced LTP was observed in male runner mice suggesting a differential sensitivity to exercise in adolescent female and male mice, possibly due to differences in development. However, in male and female adult and aged mice the effects are generally positive. Five weeks (Wu et al. 2008) and 8 months of forced exercise on a treadmill (O'Callaghan et al. 2009) as well as voluntary exercise (Marlatt et al. 2012) restored reduced BDNF and TrkB levels in the DG of middle-aged mice (Hattiangady et al. 2005; Wu et al. 2008; O'Callaghan et al. 2009). This effect correlates with enhanced neurogenesis and improvement of cognitive function (Wu et al. 2008; O'Callaghan et al. 2009; Marlatt et al. 2012). Effects of exercise on proBDNF levels, important for synaptic plasticity (Ding et al. 2006) remain to be determined.

In addition to BDNF, other neurotrophins, such as fibroblast growth factor 2 (FGF-2) (Gomez-Pinilla et al. 1997) and nerve growth factor (NGF) (Neeper et al. 1996) show a marked upregulation of mRNA in the hippocampus as a result of exercise. However, the increase in these factors is transient and less pronounced than exercise-induced increases in BDNF. In particular, upregulation of FGF-2

mRNA level was found after four nights of running and only observed in the hippocampus (Gomez-Pinilla et al. 1997). Similarly, NGF mRNA increased after the second night of running, mainly in the DG and hilus (Neeper et al. 1996). Furthermore, 8 weeks of treadmill exercise restored NGF mRNA levels in the hippocampus of aged rats (Chae and Kim 2009), as well as long-term forced exercise (8 months) in the DG of middle-aged mice (O'Callaghan et al. 2009). Both FGF-2 and NGF, have been implicated in adult neurogenesis. Using mice genetically deficient in FGF-2 (FGF-$2^{-/-}$ mice), intraventricular application of FGF-2 by viral gene transfer showed increased proliferation of progenitor cells in the DG (Yoshimura et al. 2001). Similarly, intracerebroventricular infusion of FGF-2 has been found enhance the neurogenesis and the dendritic complexity of the newborn granule cells (Rai et al. 2007). In a recent study, it was shown that FGF-2 deficiency in mice (FGF-$2^{-/-}$ mice) does not produce alterations in cell proliferation, but causes defects in neural differentiation in the adult DG. This defect was not rescued by exogenous FGF-2. Neutralization of FGF-2 with an antibody to FGF-2 did not interfere with neurogenesis, suggesting that FGF-2 may operate synergistically in combination with other mechanisms/growth factors to mediate the maturation of new neurons in the adult DG (Werner et al. 2011).

Other trophic factors that have been shown to be regulated by exercise and influence adult neurogenesis include vascular endothelial growth factor (VEGF) and insulin like growth factor I (IGF-I). Recent evidence indicates that VEGF can act as a neurotrophic factor (Ogunshola et al. 2002) and produces neurogenic effects on progenitor cells (Jin et al. 2002; Cao et al. 2004). Interestingly, it has been shown adult hippocampal neurogenesis occurs near the local microvasculature of hippocampus (Palmer et al. 2000; Fabel et al. 2003). Angiogenic changes associated with exercise have been shown to occur in the hippocampus (Fabel et al. 2003; van Praag et al. 2005; Clark et al. 2009; Van der Borght et al. 2009) that may be mediated by VEGF. Indeed, VEGF is a hypoxia-inducible protein that promotes angiogenesis through receptor tyrosine kinases on endothelial cells (Krum et al. 2002). Fabel and colleagues (Fabel et al. 2003) have shown that blockade of peripheral VEGF inhibits the increase in neurogenesis observed with running. Moreover, it has been shown that 50 days of exercise increases neurogenesis, density of blood vessels in the DG, but not other hippocampal subfields, and enhances performance in the water maze (Clark et al. 2009). Changes in neurogenesis and angiogenesis can be observed as early as 3 days of running (Van der Borght et al. 2009). Changes in cerebral blood volume measured using MRI imaging have been shown to be correlated with enhanced neurogenesis in exercising mice; these changes were specific to the DG. As in mice, exercise increased cerebral blood volume in humans and correlated with cognitive function, suggesting that this measurement may be an indirect measure for levels of neurogenesis in humans (Pereira et al. 2007). However, it should also be noted that an increase in angiogenesis is not necessarily linked to increased neurogenesis (van Praag et al. 2007).

Vasculature changes associated with exercise in the adult brain are also mediated by IGF-1 (Lopez–Lopez et al. 2004). Exercise enhances IGF-1 levels in the periphery (Trejo et al. 2001) and brain (Carro et al. 2000). At least part of the increase in the brain reflects elevated transport from the periphery across the blood–brain barrier (Reinhardt and Bondy 1994). It has been shown that systemic injection of IGF-1 in sedentary rats mimics the effects of exercise, including enhanced neurogenesis, patterns of neuronal accumulation of IGF-1, c-Fos and BDNF expression in the hippocampus, while subcutaneous infusion of a blocking IGF-1 antibody produces the opposite effects (Carro et al. 2000; Trejo et al. 2001). Peripheral infusion of IGF-1 also increased adult neurogenesis (Aberg et al. 2000), and reversed the aging related reduction in new neuron production (Lichtenwalner et al. 2001). Because IGF-1 induced-modifications correlated with BDNF levels, it has been suggested that BDNF may be a potential downstream target that mediates some of the protective effects of IGF-1 (Ding et al. 2006). In addition, IGF-1 is able to increase the spontaneous firing in neurons accumulating IGF-1; therefore, peripheral IGF-1 may initiate growth factor cascades in the brain that can alter synaptic plasticity.

5 Exercise and Neurotransmitters

Physical activity influences many neurotransmitter systems in the brain including the glutamatergic (Farmer et al. 2004; Kitamura et al. 2003; Lou et al. 2008; Vasuta et al. 2007), GABAergic (Molteni et al. 2002), endocannabinoid (Hill et al. 2010), opioidergic (Sforzo et al. 1986), and monoaminergic systems (Chaouloff 1989). One of the main components of the glutamatergic system that has been linked to neurogenesis and synaptic plasticity is the NMDA receptor. The expression of the NR2A and NR2B subunits of this receptor were found to be significantly increased after physical activity (Farmer et al. 2004; Kitamura et al. 2003; Lou et al. 2008; Vasuta et al. 2007). However, genes related to the gamma-aminobutyric acid (GABA) ($GABA_A$ receptor, glutamate decarboxylase GAD65) system were downregulated after physical activity (Molteni et al. 2002). Knowledge of the role of the AMPA receptor in exercise induced plasticity is limited, though a recent study revealed exercise-induced changes to the AMPA receptor subunits, GluR1 and GluR2/3 (Real et al. 2010).

The psychological changes associated with prolonged physical activity are often described as a ‘runner’s high’. Exercise induced changes in psychological functions are frequently reported to being as a direct consequence of alterations in the endogenous opioid system (Schwarz and Kindermann 1992). More recently, an alternative hypothesis to explain this phenomenon was proposed, showing that exercise increases blood concentrations of endogenous endocannabinoids (Heyman et al. 2012). Voluntary wheel running increased the agonist binding site density of the cannabinoid CB(1) receptor, CB(1) receptor-mediated GTPgammaS binding, and the levels of anandamide in the hippocampus (Hill et al. 2010). These alterations were required for the exercise-induced increase in progenitor cell

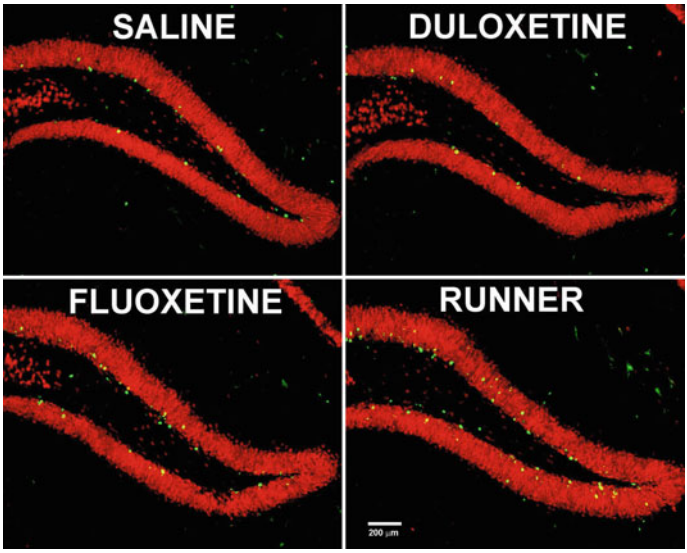

Fig. 3 Running but not antidepressants enhances neurogenesis. Photomicrographs of the dentate gyrus in sections derived from mice injected with saline, duloxetine 6 mg/kg, fluoxetine 18 mg/kg, or housed with a running wheel for 28 days. BrdU labeled cells (*green*) and cells labeled with the neuronal marker NeuN (*red*). Co-labeling analysis indicated that running mice had more than 2-fold increase in the number of new neurons compared to all the other groups (Marlatt et al. 2010)

proliferation in the hippocampus (Hill et al. 2010), and may play a role in the well-documented antidepressant effects of exercise (for review see Ota and Duman 2012). Indeed, the changes in monoamines with exercise are of particular interest as physical activity has been shown to lead to recovery from depression (Lawlor and Hopker 2001). The antidepressant effect of exercise for mild depression in humans (Ernst et al. 2006) has been shown to be just as potent as that of serotonergic medications (Babyak et al. 2000). Therefore, it is of interest that serotonergic agonists, including antidepressants such as fluoxetine (Encinas et al. 2006; Malberg et al. 2000), have been suggested to enhance cell genesis, whereas administration of the serotonin 5-HT (1A) receptor antagonists, decreases cell proliferation in the DG (Radley and Jacobs 2002).

In a recent study, we aimed to evaluate to what extent the effects of antidepressants on neurogenesis are comparable to those of voluntary wheel running. Specifically, neurogenesis was evaluated in 2-month-old female C57Bl/6 mice after 28 days of treatment with either fluoxetine, a selective serotonin reuptake inhibitor which has been shown to enhance neurogenesis (Santarelli et al. 2003), duloxetine, a dual serotoninergic-noradrenergic reuptake inhibitor, or exercise. Interestingly, only exercise enhanced (by 200 %) the BrdU positive cell survival. In addition, only fluoxetine and exercise resulted in a phenotype shift with a greater percentage of BrdU-positive cells becoming new neurons (Fig. 3). Thus, the neurogenic response to exercise is much stronger than to antidepressants, and it

is not very likely that anxiolytic effects of these drugs are mediated by adult neurogenesis (Marlatt et al. 2010). Recent research by others supports our findings in this regard (Hanson et al. 2011).

6 Endurance Factors

Another logical approach toward identifying pharmacological ways to enhance neurogenesis is the investigation into whether skeletal muscle activation as a result of exercise or pharmacological agents underlies neurogenic and cognitive effects of aerobic activity. Indeed, much research pertaining to the effects of exercise on brain function has focused on cellular, structural, and biochemical changes in the brain without much consideration for the peripheral factors that may elicit changes in synaptic plasticity, angiogenesis, neurogenesis, and cognition (Cotman et al. 2007; Gomez-Pinilla et al. 2008; Hillman et al. 2008; van Praag 2008). Physical activity has also shown effects on neurotropic factors in mammalian skeletal muscle (for review see Sakuma and Yamaguchi 2011), an abundant source of neurotrophins (Chevrel et al. 2006). Increased BDNF levels and BDNF mRNA expression in the peripheral system have been described following exercise (Gomez-Pinilla et al. 2002; Allard et al. 2004; Ferris et al. 2007). These increments may play a role in enhancing glucose metabolism and may act as a myokine, producing neurotrophic effects in the brain (Sakuma and Yamaguchi 2011). Indeed, the possibility that skeletal muscle activation as a result of exercise or pharmacological agents underlies cognitive effects of aerobic activity has just begun to be explored.

Recently, transcriptional factors regulating muscle fiber contractile and metabolic genes have been identified (Wang et al. 2004) and led to the identification of compounds that can increase the ability of cells to burn fat and enhance exercise endurance (Narkar et al. 2008). The peroxisome proliferator activated receptor delta (PPARδ) is a transcription factor that regulates fast-twitch muscle fiber contraction and metabolism. Overexpression of this factor increased oxidative muscle fiber number. In addition, administration of the selective agonist GW501516 increased exercise stamina when combined with training (Narkar et al. 2008). PPARδ is controlled by AMP-activated protein kinase (AMPK), a master metabolic regulator important for glucose homeostasis, appetite, and exercise physiology (Hardie 2004). Treatment with AMPK agonist AICAR enhanced running endurance by 45 % in sedentary mice (Narkar et al. 2008).

We investigated the effects of endurance factors, PPARδ agonist GW501516 and AICAR, activator of AMPK on memory and neurogenesis. Specifically, mice were injected with GW or AICAR for 7 days and concurrently with BrdU to label dividing cells. Both AICAR and GW improved retention of spatial memory in the Morris water maze. Moreover, AICAR significantly and GW modestly elevated DG neurogenesis. Thus, pharmacological activation of skeletal muscle may mediate cognitive effects of aerobic exercise (Kobilo et al. 2011b) and provide a

possible therapeutic approach for conditions in which exercise is limited. Interestingly, although these compounds mimic some of the results of exercise their effects are not identical to physical activity itself. Prolonged administration of AICAR (14 days) no longer enhances spatial memory function or neurogenesis (Kobilo et al. 2011b). Similarly, short-term AICAR treatment promoted sirtuin 1 protein expression in skeletal muscle whereas 14 days of treatment did not (Suwa et al. 2011).

7 Conclusions

While previous studies suggested that both EE and exercise increase adult neurogenesis, recent research has shown only exercise enhances the production of new DG neurons. The positive effects of exercise are likely the result of a combination of factors including, but not limited to enhanced neurogenesis, modifications in synaptic plasticity, spine density, neurotrophins, and angiogenesis that may mediate the beneficial effects on learning and memory, reduction of the risk of neurodegenerative diseases and delay age-related cognitive decline. Within the hippocampus, the most pronounced changes with physical activity are in the DG subfield, with an increased production of new neurons and BDNF levels, which are associated with improved performance on tasks presumably mediated by the DG. Furthermore, running, but not antidepressants that block serotonergic or noradrenergic reuptake, triggers the production of new neurons in the DG. However, we also suggest that caution should be used when applying exercise to conditions of brain injury and neurodegenerative disease as the consequences could be detrimental as found in a mouse model of Huntington's disease. While further research is needed to understand the cellular mechanisms underlying effects of aerobic activity on the brain, exercise is a powerful lifestyle intervention that could be used to augment and maintain cognitive function throughout the lifespan.

Acknowledgments This work was supported by the National Institute on Aging, Intramural Research Program.

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The Effect of Systemic Chemotherapy on Neurogenesis, Plasticity and Memory

Peter Wigmore

Abstract Chemotherapy has been enormously successful in treating many forms of cancer and improving patient survival rates. With the increasing numbers of survivors, a number of cognitive side effects have become apparent. These have been called "chemobrain" or "chemofog" among patient groups, who describe the symptoms as a decline in memory, concentration and executive functions. Changes which, although subtle, can cause significant distress among patients and prevent a return to the quality of life experienced before treatment. This cognitive side effect of chemotherapy was not anticipated as it had been assumed that chemotherapy agents, administered systematically, could not cross the blood–brain barrier and that the brain was therefore protected from their action. It is now realised that low concentrations of many chemotherapy agents cross the blood–brain barrier and even those that are completely prevented from doing so, can induce the production of inflammatory cytokines in peripheral tissues which in turn can cross the blood–brain barrier and impact on the brain. A large number of patient studies have shown that cognitive decline is found in a proportion of patients treated with a variety of chemotherapy agents for different types of cancer. The deficits experienced by these patients can last for up to several years and have a deleterious effect on educational attainment and ability to return to work. Imaging studies of patients after systemic chemotherapy show that this treatment produces structural and functional changes in the brain some of which seem to persist even when the cognitive deficits have ceased. This suggests that, with time, brain plasticity may be able to compensate for the deleterious effects of chemotherapy treatment. A number of mechanisms have been suggested for the changes in brain structure and function found after chemotherapy. These include both central and peripheral inflammatory changes, demyelination of

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Curr Topics Behav Neurosci (2013) 15: 211–240
DOI: 10.1007/7854_2012_235

Published Online: 14 December 2012

white matter tracts, a reduction in stem cell proliferation in both the hippocampal neurogenic region and by oligodendrocyte precursors as well as changes in hormonal or growth factor levels. A number of possible treatments have been suggested which range from pharmacological interventions to cognitive behavioural therapies. Some of these have only been tested in animal models while others have produced varying degrees of improvement in patient populations. Currently, there is no recognised treatment and a greater understanding of the causes of the cognitive decline experienced after chemotherapy will be key to finding ways of preventing or treating the effects of chemobrain.

Contents

1 Inroduction

Given that plasticity and neurogenesis in the adult brain are both inherently responsive to environmental factors, it is not surprising that they are impacted by cytotoxic drugs. This is significant, as a number of commonly used medications with cytotoxic effects are reported to have cognitive side effects which could be explained by functional or structural changes in brain plasticity. One of the most widely recognised groups of drugs suggested to have a deleterious effect on cognition are chemotherapy agents used in the adjuvant treatment of many cancers. Chemotherapy has proved to be invaluable in the treatment of many cancers but with increasing numbers of patients surviving for prolonged periods,

increasing concern is being shown about the long-term side effects of such treatments. While the toxic effects of direct application of chemotherapy to the brain, via intrathecal or intraventricular routes have long been recognised, numerous reports now suggest that systemic chemotherapy, given in the treatment of non-brain cancers, can also affect the central nervous system and lead to long-term effects on cognition. The mechanisms behind these effects are still unclear, some chemotherapy drugs can cross the blood–brain barrier and so directly affect the brain while others induce expression of peripheral cytokines which in turn cross the blood–brain barrier and provide an indirect route by which these drugs could affect brain structure and function (Aluise et al. 2010; Blaney et al. 2004; Bourke et al. 1973; Kerr et al. 1984; Myers 2010).

2 Effect of Systemic Chemotherapy on Patients

2.1 Cognitive Studies

Investigations into the impact of chemotherapy on cognition were triggered by patient reports of mental confusion, problems at work and the inability to recall words and events after receiving this treatment. Within patient groups, the phenomena has come to be called “chemobrain” or “chemofog” and has resulted in the publication of books and pamphlets on the means of coping with these symptoms (Boykoff et al. 2009; Hardwicke 2009; Mitchell and Turton 2011; Myers 2012; Potrata et al. 2010). Initially dismissed as being the result of the stress or depression caused by diagnosis and treatment, from the early 1990s groups of mainly breast cancer patients [see also a review of ovarian cancer patients (Correa and Hess 2012)], have been psychometrically tested for specific cognitive changes which could be associated with systemic chemotherapy. These investigations have found that a proportion of patients did indeed suffer from deficits in verbal memory, executive function and concentration after receiving chemotherapy. The severity of the effects was dose dependent and could persist for a number of years after the cessation of treatment (Ahles et al. 2002, 2005; Brezden et al. 2000; Castellon et al. 2004; de Ruiter et al. 2011b; Gottschalk et al. 2003; Jim et al. 2009; Koppelmans et al. 2012; Kreukels et al. 2008b; Mar Fan et al. 2005; Reid-Arndt et al. 2010; Schagen et al. 2002; Scherwath et al. 2006; Tchen et al. 2003; van Dam et al. 1998; Weis et al. 2009; Wieneke and Dienst 1995; Yamada et al. 2010). These studies were cross sectional in design and compared patient cognitive performance with either normative population values, patients not treated with chemotherapy or age and education matched healthy individuals. Use of this type of control population has a number of possible confounding problems due to the differences in experience between control and chemotherapy treated groups. As a result, prospective longitudinal studies have largely superseded these cross-sectional investigations. With this paradigm, patients are cognitively tested

before the start of chemotherapy and at various time points post treatment. Although not without its own methodological difficulties these studies produce baseline scores for each patient to compare with later time points. Using this approach, a surprising result was that up to 20 % of patients have cognitive impairments, when compared with population norms, prior to the start of chemotherapy. The cause of this is still unknown but may be related to either the stress experienced by patients or as a direct, possibly inflammatory effect of the cancer. Support for these theories has come from animal models bearing peripheral tumours that show increased brain cytokine levels and behavioural deficits (Pyter et al. 2009, 2010). In addition, fMRI studies have also found differences between cancer patients prior to chemotherapy and healthy controls (Cimprich et al. 2010; Scherling et al. 2012a, b).

However, even when reduced pre-treatment cognitive scores are taken into account, most longitudinal studies have still found that a subset of between 10 and 45 % patients show a cognitive decline associated with the period of chemotherapy (Ahles et al. 2010; Bender et al. 2006; Biglia et al. 2012; Collins et al. 2009; Deprez et al. 2011, 2012; Galalae et al. 2005; Hedayati et al. 2012; Hermelink et al. 2007; Hess et al. 2010; Hurria et al. 2006; Jansen et al. 2011; Quesnel et al. 2009; Schagen et al. 2006; Vearncombe et al. 2009, 2011; Wefel et al. 2004, 2010; Whitney et al. 2008). Patients with low cognitive scores prior to drug treatment seem particularly susceptible to decline after chemotherapy, suggesting that patients with a high level of cognitive reserve are more resistant to the effects of chemotherapy in comparison to those with less structural (brain) or functional (cognitive) reserve (Ahles et al. 2010). Chemotherapy using cytotoxic drugs is now often supplemented by endocrine therapy in cases of oestrogen or progesterone receptor-positive breast cancers. These treatments are designed to reduce oestrogen production [aromatase inhibitors; (AI)] or to bind to oestrogen receptors [selective oestrogen receptor modulators; (SORM)]. Animal and human studies have demonstrated the importance of oestrogen for cognition (Brann et al. 2007). Its actions include increased dentritic branching, hippocampal neurogenesis and enhanced LTP. Studies of patients who have taken endocrine therapy have mostly found a reduction in cognition particularly with tamoxifen, a SORM, but also with AIs (Ahles et al. 2010; Bender et al. 2007; Jenkins et al. 2004; Paganini-Hill and Clark 2000; Phillips et al. 2011; Schilder et al. 2010); however, other studies find no affect (Hermelink et al. 2008). A recent animal study of the cognitive effects of tamoxifen found a cognitive decline (Walker et al. 2011). Larger scale patient studies have however produced conflicting results reviewed in (Agrawal et al. 2010) and further studies are needed to resolve this issue.

It is worth noting that not all studies of the cognitive effects of chemotherapy have found impairments after treatment (Debess et al. 2010; Donovan et al. 2005; Galica et al. 2012; Hermelink et al. 2010; Jenkins et al. 2006; Kurita et al. 2011; Mehlsen et al. 2008; Tager et al. 2010). The reasons for this are unclear but may relate to the psychological tests used together with the type of cancer and treatment received.

2.2 Imaging Studies

Cognitive testing has been supplemented by neuro imaging studies of cancer survivors who have been treated with chemotherapy. Some structural MRI investigations have shown a reduction in either total brain volume (Koppelmans et al. 2011) or a reduction in specific regions including prefrontal and parahippocampal areas (Inagaki et al. 2007) while others have failed to find differences in hippocampal volumes (Yoshikawa et al. 2005). A longitudinal study, where patients were scanned prior to and after chemotherapy, showed reductions in frontal cortex, temporal lobes and cerebellar grey matter 1 year after treatment but recovery by most areas within 4 years (McDonald et al. 2010). An fMRI study also found changes in frontal cortex activation which appeared to return to baseline values by 1 year (McDonald et al. 2012). However, another recent study has found changes in brain networks indicating a reduced ability to process information which appeared to worsen with time (Bruno et al. 2012).

Particular interest has been shown in the impact of chemotherapy on brain white matter tracks. Diffusion tensor imaging shows increased brightness in these tracks after chemotherapy, interpreted as being due to demyelination or axonal damage (Abraham et al. 2008; Akitake et al. 2011; de Ruiter et al. 2011a, b; Deprez et al. 2011, 2012). Animal studies have similarly found that MTX causes a reduction in the density of the corpus callosum (Seigers et al. 2009) and direct evidence for demyelination after 5-Fluorouracil (5FU) treatment (Han et al. 2008). Post chemotherapy fMRI and PET imaging has also shown alterations in blood flow and metabolism during cognitive tasks, suggesting that in comparison with non-chemotherapy patients, treated patients have to use increased areas of the brain to complete cognitive processes (de Ruiter et al. 2011b; Ferguson et al. 2007; Kesler et al. 2009, 2011; Silverman et al. 2007).

2.3 Duration of Effects

Of particular interest to patients is the duration and likelihood of recovery from any cognitive deficits experienced after chemotherapy. Measurement of recovery has varied between studies with most studies finding cognitive changes within the first year post chemotherapy and where testing has been carried out beyond this, detecting improvements with time. Many studies find no significant differences from controls between 1 and 5 years post treatment (Collins et al. 2009). A few studies however have detected deficits 10 years after treatment (de Ruiter et al. 2011b; Koppelmans et al. 2012; Yamada et al. 2010). Similarly, while some imaging studies have also found improvements with time (McDonald et al. 2010) a number still detect changes 10–20 years post treatment (de Ruiter et al. 2011a, b; Koppelmans et al. 2011; Silverman et al. 2007), or no improvement with time (Inagaki et al. 2007). Although these results need confirmation, they suggest that

chemotherapy may produce permanent changes to brain structure but that functional recovery after a number of years is still possible in most cases.

2.4 Animal Studies

The deleterious cognitive effects of chemotherapy drugs have been confirmed in a large number of animal studies. Recent papers are shown in Table 1 and earlier papers are reviewed in (Lyons et al. 2011a; Seigers and Fardell 2011; Wigmore et al. 2010). Investigations have shown that rodents treated, generally with single chemotherapy agents (5FU; MTX; Cyclophosphamide; Doxorubicin; Oxaliplatin; thioTEPA; Cytosine arabinoside; Temozolomide; BCNU; Cisplatin; Cytarabine; Paclitaxel) as well as other cytotoxic drugs (methylazoxymethanol acetate (MAM); 3′-azido-deoxythymidine (AZT)), show a decline in their ability to perform a number of behavioural tasks. These include spatial navigation (Morris Water maze) particularly if this requires the necessity of flexibility in learning; the recognition of novelty (novel object location and recognition tasks); the association of context with a stimulus (contextual fear conditioning) and operant response learning. These behavioural changes can be interpreted as deficits in hippocampal and frontal cortex functions and appear to reproduce a number of the cognitive deficits described by patients. In some studies, specific cognitive deficits can be correlated with a reduction in neurogenesis together with changes in long-term potentiation (LTP) and conduction (Gandal et al. 2008; Garthe et al. 2009; Han et al. 2008).

Different chemotherapy agents acting in different ways can produce similar cellular and cognitive changes indicating that they appear to be causing a general neuro toxicity which is not specific to particular drugs (Rzeski et al. 2004). In vivo studies have been complemented by those in vitro which have shown differential sensitivity to chemotherapy agents by neural stem cells and other cell populations which may explain some of the effects of treatment (Dietrich et al. 2006; Wick et al. 2004). Relatively few studies have looked at inflammation, corticosteroid levels or changes in neurotransmitter levels which could affect cognition and this is an underexplored area.

In conclusion, systemic chemotherapy appears to produce mild to moderate cognitive deficits in a proportion of patients. Relatively little attempt has been made to associate particular cognitive changes with specific chemotherapy drugs and most patients are treated with a combination therapy containing a number of drugs. However, 5FU and methotrexate (MTX), both commonly used in the treatment of breast cancer, are agents frequently associated with “chemobrain” and are associated with higher levels of toxicity. Cognitive changes are accompanied by structural and functional changes to the brain as shown by imaging, which may persist and have been described as being equivalent to 4 years of additional ageing (Koppelmans et al. 2011).

Table 1 Recent animal models examining the cognitive effects of chemotherapy drugs

Wilson and Weber (2013)	thioTEPA	Mouse	Long-term increase in depressive behaviours Reduced cell proliferation in dentate gyrus
Christie et al. (2012)	Cyclophos Doxorubi	Rat	Cognitive deficits Reduced number of DCX-positive cells Reduced neurogenesis
ELBeltagy et al. (2012)	5FU	Rat	Long-term reduction in cell proliferation in dentate gyrus
Fardell et al. (2012)	5FU Oxaliplatin	Rat	Cognitive impairments with combined drugs, prevented by wheel running
Fremouw et al. (2012a)	Doxorubi Cyclophos 5FU	Mouse	No cognitive effects
Fremouw et al. (2012b)	AraC	Mouse	No cognitive effects
Lyons et al. (2012)	5FU	Rat	Cognitive impairments; reduction in cell proliferation in the dentate gyrus. Effects prevented if Fluoxetine given before and during chemotherapy
Sharpe et al. (2012)	Oxaliplatin	Rat	Cognitive deficits
Winocur et al. (2012)	5FU	Mouse	Persistent cognitive deficits
Yang et al. (2012)	MTX	Mouse	Tumour and non-tumour bearing animals compared. Increase in depressive behaviours Reduced numbers of DCX-positive cells Increased levels of iNOS and COX2
Briones and Woods (2011)	Cyclophos MTX 5FU	Rat	Cognitive deficits Reduced cell proliferation in dentate gyrus Reduced HDAC activity
Long et al. (2011)	Cyclophos 5FU	Rat	No cognitive effects 2 months later
Lyons et al. (2011b)	Cyclophos	Rat	No cognitive effects. No reduction in hippocampal cell proliferation Reduced cell survival
Lyons et al. (2011c)	MTX	Rat	Cognitive deficits and reduced cell proliferation in dentate gyrus Effects prevented by co-administration of fluoxetine
Merzoug et al. (2011)	Doxirubi	Rat	Increased anxiety and brain oxidative stress

(continued)

Table 1 (continued)

Walker et al. (2011)	MTX 5FU Tamoxifen	Mouse	Cognitive deficits which were potentiated by Tamoxifen
Winocur et al. (2011)	MTX 5FU	Mouse	Cognitive deficits produced by chemotherapy agents which were prevented by Donepezil
Yang et al. (2011)	MTX	Mouse	Cognitive deficits Increase in depressive behaviours Transient declines in cell proliferation; DCX-positive cells and apoptosis
ElBeltagy et al. (2010)	5FU	Rat	Cognitive deficits; reduced cell proliferation in the dentate gyrus Cognitive and cell deficits prevented by co-administration with fluoxetine
Fardell et al. (2010)	MTX	Rat	Long-term cognitive deficits
Imayoshi et al. (2010)	AraC	Mouse	Reduced cell proliferation
Joshi et al. (2010)	Doxirubi	Mouse	Decreased antioxidant enzymes
Li et al. (2010a)	MTX	Rat	Cognitive deficits
Li et al. (2010b)	MTX	Rat	Cognitive deficits Reduced folates in CSF
Mondie et al. (2010)	thioTEPA	Mouse	Long-term cognitive deficits No effect on depressive behaviours Reduced cell proliferation in dentate gyrus
Seigers et al. (2010a)	MTX	Rat	Tumour and non-tumour bearing animals compared Reduced cell proliferation in dentate gyrus
Seigers et al. (2010b)	MTX	Rat	Reduced blood vessel density Activation of microglia
Yang et al. (2010)	Cyclphos	Mouse	Short-term cognitive deficits Transient decrease in cell proliferation and DCX-positive cells in the dentate gyrus

Dosages, routes of administration and the timing of end points vary between papers
Abbreviations: *AraC* Cytosine arabinoside, *Cyclophos* Cyclophosphamide, *Doxirubi* Doxirubicin, *5FU* 5-Fluorouracil, *MTX* Methotrexate

Animal studies confirm that chemotherapy treatment can produce cognitive decline and the similarities between patient reports of memory and concentration deficits which are dependent upon hippocampal and frontal cortical brain regions suggest that these models have clinical validity. Relatively few animal studies have looked at the long-term cognitive effects of treatment but spatial memory

deficits have been found for up to several months post treatment again mimicking patient reports of persistent effects (Li et al. 2008; Mondie et al. 2010; Winocur et al. 2012).

3 Mechanisms Behind the Effects of Chemotherapy on Cognition

The mechanisms by which systemic chemotherapy produces behavioural and cognitive changes are currently unclear and as different chemotherapy agents may impact the brain in different ways, a wide range of suggestions have been made (Ahles and Saykin 2007; Janelsins et al. 2011; Joly et al. 2012). These have sometimes been difficult to test in patient populations and so animal models of the cognitive side effects of chemotherapy have mainly been used to investigate these suggestions.

3.1 Inflammation

Chemotherapy can cause inflammatory reactions leading to elevated levels of pro inflammatory cytokines. These can be produced in peripheral tissues and subsequently cross the blood–brain barrier to affect the brain or chemotherapy may produce inflammation within the brain itself. Inflammation and the associated cytokines are known to cause cognitive decline and to reduce hippocampal neurogenesis (Bastos et al. 2008; Ekdahl et al. 2009; Hein and O'Banion 2009; Wu et al. 2012). Several chemotherapy agents (etoposide, 5FU, Doxorubicin) elevate pro inflammatory cytokine production by cells in vitro (Elsea et al. 2008) and studies of breast cancer patients have shown, at least a transient, increase in pro inflammatory cytokines by taxenes (Pusztai et al. 2004; Tsavaris et al. 2002) and Doxorubicin (Janelsins et al. 2012). The changes in cytokine levels brought about by Doxorubicin have been correlated with self-reported declines in cognition. In contrast, patients on a regime containing 5FU showed a decline in cytokine levels and no correlation with cytokine levels and cognition. MTX has anti-inflammatory properties (Hobl et al. 2011) and may not produce its cognitive effects by this mechanism. In some animal models, chemotherapy induced inflammation within the brain has not been detected after systemic delivery of 5FU (ElBeltagy et al. 2012; Han et al. 2008) while MTX while activating brain microglia did not produce a rise in inflammatory cytokine levels within the brain (Seigers et al. 2010b). However, a recent study using MTX, has detected elevated levels of the prostaglandin producing enzyme, COX-2 and nitric oxide producing enzyme iNOS suggestive of an inflammatory response (Yang et al. 2012). Peripherally delivered anthracyclines (Doxorubicin), which do not cross the blood–brain barrier, can cause a cognitive decline in animal models (Konat et al. 2008; Macleod et al.

2007). This appears to be mediated by peripheral elevation of TNF-α which can enter the brain and produce oxidative stress and focal areas of inflammation (Joshi et al. 2010). Blocking antibodies raised against TNF-α prevent these inflammatory changes (Tangpong et al. 2006) while antioxidants can prevent the cognitive changes (Konat et al. 2008). The role inflammation may play in producing the cognitive effects of systemic chemotherapy is still unclear and there is currently a lack of patient studies looking at the long-term effects of a variety of agents on cytokine levels.

3.2 Action Chemotherapy on Stem Cell Populations

All chemotherapy agents act as cytostatics, with their primary action being to block the proliferation of dividing cells. As such they would be expected to have a particularly strong effect on proliferating cell populations within the brain. Stem and progenitor cells involved in the generation of new neurons and glia may therefore be susceptible. In addition, it is becoming apparent that some non-dividing cell populations are also sensitive to chemotherapy. Two cell populations have received particular attention, oligodendrocytes and their precursors involved in maintaining myelination in white matter tracks and the neural stem cells which produce both neurons and glia in the hippocampus and subventricular zone of the lateral ventricles.

As noted above, a feature of chemotherapy is changes in the MRI appearance of white matter tracks which appears to be due to demyelination. Consistent with these observations, patients and animal models show persistent changes in conduction properties after chemotherapy indicative of white matter damage (Gandal et al. 2008; Han et al. 2008; Kreukels et al. 2008a). Animal and in vitro studies show that both mature oligodendrocytes and their precursors can be killed or prevented from dividing by chemotherapy (Han et al. 2008). White matter tracts have a turnover of glial cells and loss of both oligodendrocytes and their progenitors is likely to cause demyelination in these regions. An important observation is that the reduction in oligodendrocyte precursor proliferation after 5FU treatment can be delayed but persists for at least 6 months post drug treatment (Han et al. 2008). These long-term effects of 5FU on the cells required for myelination provide one mechanism for the prolonged effects of chemotherapy described by patients.

As discussed in other chapters within this volume, adult hippocampal neurogenesis generates new granule cell neurons for the dentate gyrus. These are continually produced throughout life from neural stem cells residing in a narrow region, the subgranular zone (SGZ), on the inner side of each blade of the dentate gyrus (Ming and Song 2011; von Bohlen und Halbach 2011). The SGZ provides a stem cell niche which regulates the proliferation and differentiation from neural stem cells, primarily of new neurons but also of a smaller number of astrocytes. Most neural

stem cells within the SGZ are quiescent but their division generates rapidly dividing neural progenitors (transit amplifying cells) which differentiate, over a period of several weeks, into dentate gyrus neurons (Lugert et al. 2010). The generation of these new neurons provides additional plasticity to hippocampal circuits which is required for memory (Deng et al. 2010; Inokuchi 2011; Koehl and Abrous 2011; Marin-Burgin and Schinder 2012). Increases or decreases in the production of new dentate gyrus neurons are generally correlated with similar changes to cognitive functions in particular the consolidation of short- into long-term memories.

Adult hippocampal neurogenesis is responsive to environmental influences and like oligodendrocyte precursors, the proliferating neural stem and progenitor cells within the SGZ of the dentate gyrus are particularly sensitive to systemic chemotherapy. A large number of animal studies, reviewed in (Lyons et al. 2011a; Seigers and Fardell 2011; Wigmore et al. 2010) have shown that all chemotherapy drugs tested so far, reduce cell proliferation in the SGZ. This results in a reduction in the number of new neurons being added to the dentate gyrus which in turn is associated with the cognitive impairments described above. As many of the patient descriptions of "chemobrain" include aspects of memory which require hippocampal functions, inhibition of hippocampal neurogenesis is one of the prime candidates for the causes of this aspect of cognitive decline after chemotherapy.

It is not surprising that the neurogenic regions of the brain, which contain large numbers of dividing neural stem and progenitor cells, are highly susceptible to anti-mitotic cancer treatments including radio and chemotherapy. Less easy to explain is the persistent reduction in cell proliferation found after some chemotherapy regimens. The response of stem cell populations to chemotherapy, however, may be dependent upon the nature and duration of the treatment. Single drug treatments can lead to an immediate but transient drop in cell proliferation followed by a rebound to normal levels within several days (Mondie et al. 2010; Yang et al. 2010, 2011). In contrast, multiple injections of chemotherapy agents over a period, or single high doses, can cause a prolonged and progressive reduction in the proliferation which continues post treatment (ElBeltagy et al. 2012; Han et al. 2008; Mondie et al. 2010).

The observed effects of chemotherapy on neural progenitors are similar to the impact of these drugs on cell proliferation in the haematopoietic system. Here, multiple treatments with chemotherapy lead to prolonged myeloid depression and the subsequent failure to generate new blood cells, a dose limiting effect of the treatment. The haematopoietic system, within the bone marrow, shows similarities to the generation of neurons in the hippocampus and has at its base a population of largely quiescent stem cells which divide to produce a rapidly proliferating progenitor population. Initial treatment with chemotherapy primarily affects this rapidly dividing population and leads to a significant drop in the numbers of dividing cells. Quiescent stem cells, which largely escape damage by the initial dose of chemotherapy, are activated to start dividing so that the bone marrow is repopulated (Lerner and Harrison 1990; Randall and Weissman 1997; Wang et al.

2006). Further treatment with chemotherapy, within a few days of the initial application, affects these dividing stem cells, reducing their number but also changing their behaviour so that they become quiescent. The mechanism behind this induced replicative senescence or quiescence is unclear but may be a reversible form of senescence in which cells which have ceased to divide may subsequently re-enter mitosis (Campisi and d'Adda di Fagagna 2007; Gewirtz et al. 2008). Induction of this state is brought about after detection of DNA damage and stabilisation of the transcription factor p53. A downstream target of p53 is the cyclin-dependent kinase inhibitor p21 and upregulation of p21 causes cells to arrest at the G1/S point in the cell cycle. DNA damage can be produced by the direct effects of chemotherapy on the process of DNA synthesis, induction of DNA strand breaks or by damage to mitochondria leading to the production of reactive oxygen species and oxidative damage to DNA. Quiescence after chemotherapy enables cells to avoid apoptosis, which would be a consequence of entering mitosis with DNA damage, and to undertake DNA repair and subsequently divide. Induction of quiescence has been demonstrated after treatment with a number of different chemotherapy drugs (Akatsu et al. 2007; Dabrowska et al. 2009, 2011). In some cases senescence or quiescence is produced by low doses of chemotherapy, insufficient to cause apoptosis, but similar to the levels of chemotherapy drugs found in the brain after systemic administration (Huang et al. 2010; Rebbaa et al. 2006; Zhang et al. 2011). This scenario is consistent with the transient apoptosis but prolonged reduction in neurogenesis is seen after MTX and 5FU treatment (ElBeltagy et al. 2012; Han et al. 2008; Yang et al. 2011). Senescent cells have been found to release pro inflammatory cytokines (Dabrowska et al. 2011) linking the process of senescence to the effects of inflammation discussed above.

Epigenetics is the process by which non-coding modifications to DNA and its associated histone proteins can modulate gene expression. These effects are now known to regulate the progress of neurogenesis in the adult brain, in particular controlling the number of cells produced and their differentiation programme (Mohamed Ariff et al. 2012; Sun et al. 2011). It has recently been shown that a number of chemotherapy drugs have epigenetic effects. For example when low doses of Doxorubicin and MTX induce cell cycle arrest and senescence, this is caused by the inhibition of histone deacetylase enzymes and the increased acetylation of the p21 promoter (Huang et al. 2011; Rebbaa et al. 2006). Rats treated with a cyclophosphamide, MTX and 5FU (CMF) regime for 4 weeks have reduced cell proliferation in the hippocampus and impairments in spatial learning (Briones and Woods 2011). These changes to neurogenesis can be correlated with an increase in the acetylation of histone H3 and a decrease in histone deacetylase activity, a result similar to that found by Huang et al. after treatments with MTX. Histone acetylation may be a general mechanism to block cell proliferation during neurogenesis as it has also been found after Valproic acid treatment (Umka et al. 2010).

3.3 Action of Chemotherapy Drugs on the Stem Cell Environment

Although neural stem cells are found throughout the adult brain, their division and differentiation into neurons is limited to a few areas, in particular the subgranular zone of the dentate gyrus within the hippocampus and the subventricular zone of the lateral ventricles. The restricted locations in which these cells produce new neurons has led to the concept of the "stem cell niche" consisting of an environment which permits and stimulates stem cell division and the differentiation of new neurons (Riquelme et al. 2008). Important features of stem cell niches which influence neurogenesis are the astrocytic glial cells found there, the micro vasculature and the levels of growth factors produced. Interference with these aspects of the local environment of stem cells could negatively impact hippocampal neurogenesis and so cognition. Many proliferating stem and progenitor cells in the subgranular zone are closely associated with capillaries (Palmer et al. 2000). The endothelial cells lining these blood vessels secrete a number of growth factors, the most important of which are VEGF and BDNF, which are required for the survival and differentiation of neural precursors (Goldman and Chen 2011; Schanzer et al. 2004; Shen et al. 2004). Chemotherapy treatments can have significant negative effects on blood vessels within the hippocampus. Both MTX and 5FU cause endothelial cell apoptosis and a reduction in capillary density (Han et al. 2008; Seigers et al. 2010b) while 5FU reduces hippocampal BDNF levels (Mustafa et al. 2008). The impact of chemotherapy on the micro environment of stem cells within the hippocampus may therefore contribute to the reduction in hippocampal neurogenesis and the negative effect of chemotherapy on cognition.

3.4 Genetic Susceptibility to Chemotherapy

As only a proportion of patients given chemotherapy experience cognitive impairment, it is possible that a genetic susceptibility in some individuals predisposes them to this outcome after treatment (Janelsins et al. 2011). The APOE $\varepsilon 4$ allele of the apolipoprotein gene has been associated with cognitive decline in breast cancer survivors (Ahles et al. 2003). APOE is a lipid binding protein whose $\varepsilon 4$ allele is associated with an increased risk of developing Alzheimer's disease and cognitive impairment after brain damage. The cause of this appears to be a breakdown in the blood–brain barrier allowing toxic substances' access to the brain which would lead to increased susceptibility to the effects of chemotherapy (Bell et al. 2012).

An additional genetic link to chemotherapy susceptibility has been found in the presence of a single nucleotide polymorphism in the catechol-O-methyltransferase gene. The effects of this polymorphism are lower levels of dopamine in the frontal cortex and poorer cognitive performance (Small et al. 2011). Breast cancer

patients with this polymorphism show impaired performance after chemotherapy in tasks requiring attention, a pre frontal cortex function.

It is possible that genetic susceptibility to the cognitive effects of chemotherapy relates to cognitive reserve, a feature defined as the resilience of the brain to damage or insult (Steffener and Stern 2012). Chemotherapy has been suggested to accelerate normal age-related cognitive decline. This effect is steeper in individuals with low cognitive reserve (Ahles 2012; Ahles et al. 2010; Maccormick 2006). A genetic predisposition to low cognitive reserve might explain why only a subset of patients show susceptibility to cognitive decline after treatment.

In conclusion, the cause of cognitive decline due to systemic chemotherapy treatment is still unclear. It is likely that a number of factors are involved and may combine to produce the long lasting effects seen in some patients. Understanding of these processes may enable effective treatments or protection of the brain during chemotherapy and has been the subject of a number of investigations (Fardell et al. 2011). The following section looks at some of the options that have been proposed.

4 Can Cognitive Decline After Chemotherapy be Prevented or Treated?

A reasonable place to start looking for a treatment for the cognitive problems experienced by patients after chemotherapy is to test drugs which have been shown to improve cognition in other clinical situations. Currently, five drug treatments have undergone either clinical trials or investigations using animal models.

4.1 Erythropoietin

Erythropoietin (EPO), a glycoprotein hormone which stimulates the production of red blood cells within the bone marrow, has been used to improve red cell counts in patients with anaemia after stroke and as a performance enhancing drug in sport (Lombardero et al. 2011). Its use in neurological conditions has received significant interest after the demonstration that it can act as a neuroprotectant after brain trauma, stroke and epilepsy (Ponce et al. 2012; Subiros et al. 2012). In these situations EPO reduces inflammation and oxidative stress as well as inhibiting apoptosis. Of relevance to the deleterious effects of chemotherapy on the brain, is the fact that EPO has been shown to improve memory in both human subjects and in animal models (Adamcio et al. 2008; Miskowiak et al. 2007). EPO improves hippocampal-dependent memory and enhances hippocampal plasticity and long-term potentiation (LTP), effects which last for at least several weeks after treatment.

A number of human trials on the impact of EPO on the effects of chemotherapy-induced cognitive decline have shown promise as a possible treatment. In three of

these studies, where EPO was given during chemotherapy (Chang et al. 2004; Iconomou et al. 2008; Mancuso et al. 2006; Massa et al. 2006), significant increases in haemoglobin levels and small but statistically significant improvements in cognition were found in the weeks post treatment. These studies suggest that chemotherapy-related anaemia may contribute to cognitive decline and increasing haemoglobin levels with EPO may alleviate this. The relatively small sample sizes, lack of placebo treated controls and the possibility of cognitive improvements due to a practice effect must, however, be taken into account when assessing these results.

Two larger studies have been carried out, one a double blind study comparing EPO treated patients with placebo treated controls. Cognitive testing before and at the end of chemotherapy as well as 6 months later found improvements in haemoglobin and quality of life in the EPO treated group from baseline at both later time points but an improvement in cognition only at the earlier time which may indicate only a transient protection of cognitive abilities (O'Shaughnessy et al. 2005). Unfortunately, more of the EPO group in this study were treated with taxenes than those in the non-EPO group making interpretation difficult.

In the second trial, comparison between EPO treated patients and those receiving standard care showed no effect on cognition at 12–30 months (Fan et al. 2009). These results are disappointing in failing to show consistent effects of EPO on cognition but suffer from difficulties in identifying which patients may be susceptible to cognitive decline because of chemotherapy and variables in other aspects of their treatment.

The recent demonstration that EPO can protect white matter tracts and improve myelination, as well as increase BDNF levels and neurogenesis in the hippocampus, features at risk from chemotherapy treatment, suggest that further studies on the effects of EPO are warranted (Cho et al. 2012; Ransome and Turnley 2007). One potential problem, however, is cautions that have been placed on using EPO in some cancers due to reduced patient survival. A result thought to be due to thrombolytic problems and possible enhancement of tumour growth by EPO but which is the subject of an ongoing debate (Oster et al. 2012).

4.2 Psychostimulants

Two stimulants, methylphenidate and modafinil, both of which augment catecholamine action and have cognitive enhancing properties have been tried as treatments for chemotherapy-induced cognitive impairment. Methylphenidate, an amphetamine-like drug, which prevents re-uptake of dopamine and noradrenalin, acts as a stimulant in healthy individuals and can enhance memory (Repantis et al. 2010). Used in the treatment of attention deficit disorder, it increases attention and focus (Bidwell et al. 2011), while when given to cancer patients with hypoactive delirium, in which cognitive impairment is a feature, it improves cognition (Gagnon et al. 2005). Treatment of cancer patients also improves the symptoms of

fatigue and weakness after chemotherapy (Cueva et al. 2012) and the drug has been used as a neuroprotectant in conditions of cytoplasmic dopamine accumulation (Volz 2008). Two placebo controlled studies have tested the effects of methylphenidate, taken during chemotherapy, on cognition (Lower et al. 2009; Mar Fan et al. 2008). Despite the obvious rational for trying this cognitive enhancer, neither study found any beneficial effects of drug treatment on cognition.

Mondafinil, a drug which has affects on many neurotransmitter systems, is used to suppress sleep and improve memory in both healthy and psychiatric patient populations (Minzenberg and Carter 2008). When given to cancer patients, this drug improved wakefulness, psychomotor speed and attention (Lundorff et al. 2009). Similarly, breast cancer patients who had completed chemotherapy improved in memory and attention after 2 months of treatment with modafinil (Kohli et al. 2009). Although both studies showed improvements in memory and attention, it is unclear whether they were compensating for chemotherapy-induced cognitive impairment or other aspects of cancer or its treatment such as fatigue. The impact of modafinil on cell proliferation has been studied in the hippocampus of treated rats where high doses administered during a sleep period, significantly reduced cell division in the hilus and non-significantly in the neurogenic SGZ region. No effect on cell proliferation was seen when the drug was given during an awake period (Kochman et al. 2009). This was interpreted as showing that sleep deprivation produced by modafinil may be detrimental to neurogenesis and possibly cognition.

4.3 Donepezil and Fluoxetine

Two other drugs, Donepezil and Fluoxetine, both used in the treatment of psychiatric illnesses, have been tested for their cognitive enhancing effects in animal studies of chemotherapy-induced cognitive decline. Donepezil, a cholinesterase inhibitor, is currently used to treat mild to moderate cognitive impairment in patients suffering from Alzheimer's disease (Herrmann et al. 2011) and has also been tested for its ability to improve cognition in ageing individuals and after brain injury (Hernandez et al. 2006; Zhang et al. 2004). In relation to cancer treatments, Donepezil improved cognition in cranially irradiated patients (Shaw et al. 2006). In animal models, Donepezil has shown neuroprotective and anti-inflammatory actions and increases hippocampal neurogenesis by improving the survival of newly generated cells. It is also able to improve spatial memory after toxic or pharmaceutical damage (Kendall et al. 2011; Kotani et al. 2008; Meunier et al. 2006; Yoshiyama et al. 2010). Recently, Winocur and colleagues (Winocur et al. 2011) demonstrated that co-administration of Donepezil during chemotherapy treatment of mice with MTX and 5FU was able to prevent the spatial memory and executive defects produced by these agents.

The selective serotonin reuptake inhibitor, Fluoxetine, is primarily used in the treatment of depression. Work in our laboratory has shown that when Fluoxetine is

given to rats before and during chronic treatment with MTX or 5FU, it is able to prevent both the memory deficits and the reduction in hippocampal cell proliferation found after administration of these drugs (ElBeltagy et al. 2010; Lyons et al. 2011c, 2012). In contrast when given after completion of chemotherapy treatment, Fluoxetine has no beneficial effects on behaviour or neurogenesis. Chronic Fluoxetine treatment increases hippocampal neurogenesis and plasticity (Boldrini et al. 2009; Encinas et al. 2006; Kodama et al. 2004; Marcussen et al. 2008; O'Leary et al. 2009; Pechnick et al. 2011); raises BDNF levels (Castren and Rantamaki 2010) and improves cognition both in patients with mild cognitive impairments and in animal models (Gallassi et al. 2006; Horsfield et al. 2002; Mowla et al. 2007). It is also neuroprotective being able to attenuate the effects of kainic acid toxicity, and reduce inflammation and prevent apoptosis in the brain (Jin et al. 2009; Lim et al. 2009). It is unclear which of these effects is protecting the brain from the impact of systemic chemotherapy and further studies are ongoing to investigate this.

Both Donepezil and SSRI antidepressants are well tolerated and have been taken by cancer patients during chemotherapy as part of their treatment for other conditions (De Waele and Van Belle 2010; Roscoe et al. 2005), which suggests that these drugs may be usable for the treatment of chemotherapy-induced cognitive decline.

4.4 Exercise

Physical exercise confers numerous benefits to the brain including an improvement in cognition (Cotman et al. 2007) and patients experiencing "chemobrain" after chemotherapy have described the benefits of taking exercise (Myers 2012). Recently, Fardell and colleagues, using a rodent model, showed that animals receiving 5FU and oxaliplatin suffered from a post treatment decline in the performance of spatial memory tasks (Fardell et al. 2012). Providing exercise wheels for 4 weeks in the cages after the completion of chemotherapy treatment, significantly improved cognition relative to non exercising animals. A similar effect of exercise in improving cognition and hippocampal neurogenesis has been described after cranial irradiation (Wong-Goodrich et al. 2010). Exercise improves memory and hippocampal neurogenesis (van Praag et al. 2005) and the mechanism by which exercise has its effects may be similar to that found after antidepressant treatment (Baj et al. 2012; Huang et al. 2012).

4.5 Behavioural Interventions

A number of studies have applied behavioural interventions to post chemotherapy cancer patients to improve their quality of life and cognition. These have ranged from meditation to cognitive behavioural therapy and most have described benefits

to the individuals involved (Biegler et al. 2009; Ferguson et al. 2012). It is likely that these approaches are particularly suitable for some individuals and should be suggested as a means of resuming a good quality of life. Together with exercise, behavioural interventions have the advantage over pharmaceutical interventions in having no side effects or interactions with other aspects of treatment.

5 Summary

The deleterious cognitive effects produced by systemic chemotherapy are now well established and appear to be a side effect of many if not all cytotoxic drugs rather than the actions of particular types of chemotherapy. Chemotherapy causes a decline in certain types of memory, concentration and executive function in a proportion of patients. Pyschometric investigations have been supported by brain imaging studies which show changes in brain structure and function after chemotherapy and by animal studies which reproduce the cognitive effects found in patients in the absence of cancer or other aspects of treatment and diagnosis. Together, these different approaches show that chemotherapy has a potentially long-term effect on prefrontal cortex and hippocampal functions. The reason that these effects are only seen in a proportion of patients is still not clear but may relate to genetic susceptibility or the degree of cognitive reserve present and require further controlled longitudinal studies (Wefel et al. 2011). The mechanisms behind the cognitive changes described are still unknown but appear to be multifactorial—involving damage to white matter tracts including demyelination and effects on the stem cell populations responsible for hippocampal neurogenesis and oligodendrocyte turnover (Monje and Dietrich 2012). In addition, changes to the brain micro vasculature and levels of cytokines and humeral factors may impact neural plasticity and function.

A range of treatments have been suggested which have been tested in patient studies or animal models—some appear to be neuroprotective and are required to be taken during or before the chemotherapy itself while others seek to improve cognition after chemotherapy has been completed. Many need to be taken out of preclinical studies into a clinical setting before it will be clear whether they work and are suitable for patient use. A better understanding of the causes and reasons behind the susceptibility to "chemobrain" would enable more rational choices of treatment to be made.

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Part III
Diseases

Novel Insights into Depression and Antidepressants: A Synergy Between Synaptogenesis and Neurogenesis?

Francis Rodriguez Bambico and Catherine Belzung

Abstract Major depressive disorder has been associated with manifold pathophysiological changes. These include metabolic abnormalities in discreet brain areas; modifications in the level of stress hormones, neurotransmitters, and neurotrophic factors; impaired spinogenesis and synaptogenesis in crucial brain areas, such as the prefrontal cortex and the hippocampus; and impaired neurogenesis in the hippocampus. Antidepressant therapy facilitates remission by reversing most of these disturbances, indicating that these dysfunctions may participate causally in depressive symptomatology. However, few attempts have been made to integrate these different pathophysiologies into one model. The present chapter endeavors (1) to review the extant literature in the field, with particular focus on the role of neurogenesis and synaptogenesis in depression; (2) and to suggest a possible interplay between these two processes, as well as, describe the ways by which improving both neurogenesis and synaptogenesis may enable effective recovery by acting on a larger neuronal network.

Keywords Neurogenesis · Synaptogenesis · Stress · Depression · Antidepressants

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Curr Topics Behav Neurosci (2013) 15: 243–291
DOI: 10.1007/7854_2012_234

Published Online: 28 December 2012

Contents

1 Introduction: The Societal Burden of Depression

Major depressive disorder (MDD) is a widespread disorder, the lifetime prevalence of which is estimated to be as high as 16.2 % among adults in the United States, entailing a financial burden of up to US$80 billion per year (Kessler et al. 2003; Greenberg et al. 2003). Because of this, it is projected that by 2030, MDD will be the largest contributor to the worldwide burden of disease (WHO 2008). MDD is diagnosed based on the presence of long-lasting key symptom features that include low mood; inability to experience pleasure (anhedonia), and a loss of interest engaging in nearly all day-to-day activities; feelings of worthlessness, guilt, or despair; drastic changes in weight; sleep disturbance; psychomotor agitation or retardation; fatigue and anergia; diminished ability to think and concentrate; and suicidal ideation (DSM IV, American Psychiatric Association 1994). Despite advancements in the development of therapeutics for MDD, current treatment options have not fulfilled optimal efficacy. For instance, clinically used

antidepressants (ADTs) have to be continuously administered for about a month at least before improvement of symptoms could be observed (Pittenger and Duman 2008; Bambico and Gobbi 2008; Bambico et al. 2009). Also, in the largest prospective, randomized ADT efficacy study to date, dubbed STAR*D or Sequenced Treatment Alternatives to Relieve Depression, 16 % of the participants dropped out because of tolerability issues and only 36.8 % achieved remission after a first level treatment with an ADT. Furthermore, 40 % of remittances subsequently led to relapses. In general, a large proportion of remitted patients also suffer from residual symptoms that may fluctuate over time (Kessler et al. 2003; Bambico and Gobbi 2008; Bambico et al. 2009).

While the exact cause of MDD is unknown, the model that best accounts for its pathoetiology is the diathesis–stress model. This model considers that MDD is triggered by a combination of biological predispositions/vulnerabilities that build up over the course of ontogenetic development (the diathesis) and precipitating factors (such as stressful experiences) occurring later in life (Monroe and Simons 1991). The diathesis may arise from genetic factors—such as polymorphisms of genes that alter monoaminergic neurotransmission, neuroplasticity, or hypothalamic–pituitary–adrenal (HPA)/stress axis function—as well as from adverse early life experiences, including childhood abuse (Kendler et al. 2002, 2006). Even when events derive internally (such as stress hormone imbalance), a depressive episode is most frequently triggered along with an extrinsic factor. This may include prolonged exposure to minor stresses (Brown and Harris 1978; Harkness and Monroe 2006), personal loss (Farmer and McGuffin 2003; Slavich et al. 2010, 2011) and stress in the workplace (Tsutsumi et al. 2001)—experiences that are often associated with internal psychological conflict. At the neurochemical level, the vulnerability of the monoaminergic system has long been the center of investigation. The most widely accepted hypothesis pertains to the depletion of monoamine transmitters in the brain. Indeed, conventional ADTs that enhance synaptic monoamine content are the main choice for first-line pharmacotherapy. In the last couple of decades, however, there has been an increasing recognition of the inadequacy of this hypothesis, regarding it as a one-sided explanation for dysfunctions that involve multiple levels. It also fails to adequately explain treatment resistance and the slow therapeutic onset of monoamine-enhancing conventional ADTs. It is increasingly being recognized that gradual onset adaptations in intracellular molecular transduction pathways that target certain genes underlie a common therapeutic action of ADTs (Duman and Voleti 2012). These genetic targets are involved in the regulation of a myriad of neuroplasticity-related events, which serve restorative, protective, or preservative functions in limbic forebrain regions involved in the control of mood and emotion (Manji et al. 2003; Tanis and Duman 2007; Krishnan and Nestler 2008; Duman and Voleti 2012). More recent research efforts have integrated neurochemical and neuroendocrinological (HPA-stress component) levels of analyses with effects on neuroplastic processes, in explaining the pathophysiology of depressive disorders. Neuroplasticity refers to the brain's ability to reorganize itself. This is essentially achieved by synaptic structural remodeling through the formation, organization, and

maintenance of neurite components of synapses (synaptogenesis), as well as by the generation of new neurons (neurogenesis). These changes enable the organism to effectively adapt to the challenges of a constantly changing environment. There is growing evidence that MDD is closely linked to defects in neuroplasticity, specifically in brain areas integral to the different behavioral and symptomatological manifestations of this disorder, such as the prefrontal cortex (PFC) and the hippocampus. ADT treatments promote synaptogenesis or neurogenesis in these areas, and this may relate to clinical improvements or remission of symptoms. However, the interplay between neurogenesis and synaptogenesis in recovery from MDD is meagerly understood. In this chapter, we present some of the current research trends regarding the pathophysiology of MDD, with special emphasis on the role of neurogenesis and synaptogenesis, as well as their interplay in the pathogenesis of the disease and in ADT action. We explore the repercussions of impairments in these neuroplastic processes within the context of the neurotrophic, neurogenesis, and network theories, addressing some of the limitations of the classical neurochemical views, and finally endeavoring to integrate them within a comprehensive framework.

2 Neurochemical Mechanisms of Antidepressant Action

There are several chemical ADT classes available for clinical use. These include the selective serotonin reuptake inhibitors (SSRIs), norepinephrine reuptake inhibitors (NRIs), serotonin and norepinephrine reuptake inhibitors (SNRIs), tricyclic ADTs (TCAs), and monoamine oxidase inhibitors (MAOIs). In addition, a few somatic treatments are mostly under investigation [deep brain stimulation (DBS), repetitive transcranial magnetic stimulation (rTMS), and vagus nerve stimulation (VNS, FDA-approved in 2005)]. The first-generation ADTs, the MAOI iproniazid and the TCA imipramine, were discovered by serendipity. Linking their pharmacological action on the central monoamine systems to their therapeutic efficacy forged the long-held monoamine hypothesis that synaptic monoamine underactivity characterizes the major neurochemical pathophysiology of depressive disorders, and that ADTs ameliorate this deficit or augment the function of intact monoamine components (Berton and Nestler 2006; Bambico et al. 2009). Virtually all conventional ADTs ultimately increase the neurotransmission of monoamines, mainly serotonin (5-hydroxytryptamine, 5-HT), but also norepinephrine (noradrenaline); whose extracellular and synaptic levels are elevated accordingly via a number of mechanisms: by slowing down enzymatic breakdown (MAOI), by preventing reuptake into neurons (SSRI, NRI, SNRI, triple reuptake inhibitors for serotonin, norepinephrine, and dopamine), or by directly enhancing midbrain serotonergic and noradrenergic neuronal discharge or transmitter release, such as those exhibited by the nonselective alpha2-adrenoceptor antagonist mirtazapine and by 5-HT_4 receptor agonists (Bambico and Gobbi 2008; Bambico et al. 2009). ADTs also indirectly influence the function of monoamine receptors in slow

progression and in ways that eventually facilitate net electrochemical signaling within the serotonergic and noradrenergic systems. For example, inhibitory receptors located on cell bodies and dendrites of midbrain raphe serotonergic neurons (5-HT_{1A}) or locus coeruleus noradrenergic neurons (alpha2), as well as those located on serotonergic neuronal terminals (5-HT_{1B} and alpha2) could gradually be desensitized by several ADTs, leading to a disinhibition of monoamine neuronal activity. Many other ADTs have their effects on the function of postsynaptic receptors. TCAs and NRIs increase the sensitivity of postsynaptic 5-HT_{1A} receptors, such as those located in the hippocampus. Lithium, also an adjuvant to other ADT drugs, facilitates postsynaptic serotonin release by changing the property of serotonergic neurons and eventually sensitizing forebrain postsynaptic 5-HT_{1A} receptors, without influencing 5-HT_{1A} autoinhibitory receptors. Electroconvulsive therapy, one of the most potent conventional ADTs, likely produces broad and global neurochemical and bioelectrical changes, and like TCAs and NRIs sensitizes postsynaptic 5-HT_{1A} receptors (for review, see Blier and de Montigny 1999; Bambico and Gobbi 2008; Bambico et al. 2009).

3 Depression and Neuroadaptation to Stress

3.1 Moving Beyond the Monoamine Hypothesis

The monoamine hypothesis falls short in adequately explaining the slow onset of action (at least a few weeks of continuous administration) of conventional monoaminergic ADTs, while the increase in monoamine levels is observed rapidly after acute ADT administration. On the other hand, some have proposed that resultant gradual monoaminergic receptor (such as 5-HT_{1A}) desensitization and sensitization could account for this slow onset (Blier and de Montigny 1999; Bambico and Gobbi 2008; Bambico et al. 2009). Nonetheless, acute monoamine depletion studies, such as the depletion of the serotonin precursor tryptophan (tryptophan-free diet) (Delgado et al. 1990, 1999) or via alpha-methyl-paratyrosine (inhibitor of the catecholamine rate-limiting enzyme tyrosine hydroxylase) (Delgado et al. 1993; Miller et al. 1996) resulted in depressive relapse in some remitted patients, but did not produce full-blown clinical depression in healthy participants (Leyton et al. 2000). Other in vivo molecular imaging studies of specific brain receptors and transporters (Parsey et al. 2006a, b; Bhagwagar et al. 2006), cerebrospinal fluid dosage studies, and large-scale genetic association studies (Hu et al. 2007) have likewise yielded equivocal data on serotonin or norepinephrine alterations in MDD patients, and suggest that these neurotransmitters may instead be involved in producing vulnerability to MDD, rather than in the pathophysiology of depressive states directly. Some studies found serotonin alterations in MDD patients, such as reduced 5-HT_{1A} receptor binding potential in the raphe nucleus, the cingulate cortex and the insula, as well as in other cortical areas (Drevets et al. 1999, 2007; Sargent et al. 2000; Meltzer et al. 2004). But because many ADTs also

desensitize/downregulate 5-HT_{1A}, these findings point to a rather complex involvement of serotonin. Moreover, since about a third of patients are resistant to these conventional ADTs, and remitted ones have high risks of relapse, ADT-induced modifications in monoaminergic neurotransmission and receptor function may not necessarily represent final downstream mechanisms of effective therapeutic action.

3.2 Dysregulated Stress Response: A Hypothalamic-Pituitary-Adrenal Axis Feedback Hypothesis of Depression

Several alternative theories of depression with a much broader scope that goes well beyond that of the monoaminergic hypothesis have been recently developed. One of these theories proposes that MDD is the consequence of a dysregulation in the HPA axis that is responsible for the release of stress hormones such as glucocorticoids (cortisol in humans, corticosterone in rodents). Indeed, this enables to reconcile knowledge of the etiology of MDD with knowledge of the alterations in brain circuitry found in MDD. Within the HPA axis, corticotrophin releasing factor (or hormone: CRF/CRH) is released from the paraventricular nucleus (PVN) of the hypothalamus; it sequentially stimulates the pituitary to produce adrenocorticotrophic hormone (ACTH) and the adrenals to release glucocorticoids into the bloodstream. Glucocorticoids then bind to two types of receptors: the mineralocorticoid receptors (MRs) and the glucocorticoid receptors (GRs). The affinity of glucocorticoids for MR is 10 times higher than for the GRs (De Kloet et al. 1998), so that GRs are only activated when the level of glucocorticoids is high. GRs prevail in the PVN and in the pituitary (de Kloet et al. 2009): when the level of glucocorticoids is excessive, this activates negative feedback over these areas limiting the degree of activation of the HPA axis (de Kloet et al. 2005; Holsboer and Ising 2010). However, GRs and MRs are also located in limbic and cortical areas, particularly in the PFC, the hippocampus, and the amygdala. Activity of the HPA axis is further limited through a negative feedback exerted via GRs located in the PFC and in the HPC, while the opposite is found with respect to GRs in the amygdala, which further stimulate the HPA (see Ulrich-Lai and Herman 2009, for a review). Excessive or chronic glucocorticoids, as well as chronic stress induce a decrease in the number of hippocampal GR proteins and RNAs (Sapolsky et al. 1984a; Herman et al. 1995; Kitraki et al. 1999, 2004) and in the number of MRs (Sterlemann et al. 2008; Dickens et al. 2009), and elicit apoptosis, impaired hippocampal neurogenesis, retraction of apical dendrites, and spine density in pyramidal cells within the hippocampus (see Lupien et al. 2009 for a review). These lead to a deficit in the negative feedback over the HPA axis (Sapolsky et al. 1984a, b; Avitsur et al. 2001) that is mostly related to suppression of the hippocampal component (Mizoguchi et al. 2003; Surget et al. 2011). Alterations similar

to the ones observed after chronic stress are also found in MDD as patients exhibit decreased HPA negative feedback, and ADTs restore most aspects of this HPA dysregulation to normal levels (see Belzung and Billette de Villemeur 2010, for a review). Interestingly, this restoration seems required for the ability of these compounds to elicit therapeutic effects, giving credence to the claim that HPA alteration seen in MDD patients is crucial and causal. This HPA feedback dysregulation might be explained by an increase in the size of the pituitary (Krishnan et al. 1991; MacMaster and Kusumakar 2004; Macmaster et al. 2006) and adrenal glands (Amsterdam et al. 1987, Dorovini-Zis and Zis 1987; Stein et al. 1993; Szigethy et al. 1994; Rubin et al. 1995, 1996), but more importantly, by marked changes in the function of the amygdala (increased), hippocampus (decreased), and the PFC (decreased). Indeed, cortisol hypersecretion in MDD has been associated with increased metabolic activity in the amygdala and with reduced gray matter in the PFC, specifically in the rostral part of the anterior cingulate cortex (Drevets et al. 2008; Treadway et al. 2009). A reduction in hippocampal function has also been observed, and is associated with a defect in HPA feedback regulation in an animal model of depression (Surget et al. 2011). In MDD patients, structural changes are found in these three brain regions. Indeed, a reliable decrease in hippocampal volume has been described in MDD patients (Campbell et al. 2004; Videbech and Ravnkilde 2004), which is associated with a high loss of hippocampal synapses (Tata et al. 2006), suggesting a decrease in hippocampal function in MDD patients. Also, layer-specific changes in the density and size of the PFC (Rajkowska et al. 1999) have been reported that likewise suggests a decrease in the function of this structure. Finally, changes in the opposite direction are reported in the amygdala, as glucose resting metabolism is abnormally elevated, specifically in a subgroup of MDD patients displaying hypersecretion of cortisol under stress (Drevets et al. 2002).

4 Depression and Perturbed Limbic Neural Communication: The Network Hypothesis

4.1 Prefrontal and Hippocampal Hubs in a Distributed Limbic Network

The heterogeneity of behavioral and pathophysiological features of MDD, including a dysregulation in hippocampus and PFC-driven HPA negative feedback, suggests a disturbance in the information flow within a larger assembly and network of neurons, organized in multiple neuroanatomical nodes. Within this network, the amygdala, the hippocampus, and several subareas of the PFC, notably the dorsolateral area and the subgenual cingulate gyrus, may occupy major hubs involved in the internal representation of the self, the ability to regulate emotional processing, and in the active shifting away from negative thinking (see Disner

et al. 2011 for a review). In other words, one may conceive of the limbic network as a distributed structure, whose many nodes each represent different symptomatological, as well as physiological core components. The depressive state may thus be understood as to result from the disruption in the pattern of neuronal signaling within this network, ultimately upsetting the appropriation of adaptive cognitive and emotional responses to stress, including the regulation of the HPA axis (Castren 2005; Castrén and Rantamäki 2010a, b). The kind and severity of emergent symptoms constituting the depressive phenotype may therefore depend upon which microcircuits are profoundly affected. The psychological foundations of this network hypothesis have their roots in cognitive theories of psychopathology and in associative network memory models (Beck 1967; Disner et al. 2011). These ideas suggest that MDD is triggered by negative cognitive schemes, particularly involving perceptual, memory, and attentional processing (Beck 1967). The spiraling and self-reinforcing propagation of ruminative thinking, catastrophism, and hopelessness in MDD might be conceived as a negative processing bias toward emotionally negative nodes in cognitive and mood-related networks, hindering the ability to normally inhibit negative mental rumination. Depressives are thus compelled to focus on adversity and lose the ability to cope with stressful situations. Other models, on the other hand, have addressed the deficit in the processing of positive information, which causes depressives to be less sensitive to reward. The PFC, which controls executive functions, determines stress controllability, and modulates endocrine and visceromotor processes, plays a major role in the acquisition and expression of negative cognitive schemes in MDD. Within the larger limbic network, the PFC is situated within a cortico-striato-pallido-thalamic loop, whose circuits are modulated by the amygdala (involved in fear extinction) and the hippocampus (memory function). Aptly, many of these structures have been collectively called the default mode (DM) network since they are normally active during self-referential and resting states, and become less active during nonself-referential, goal-directed engagements (Price and Drevets 2010, 2012; Hamilton et al. 2011). Among MDD patients, however, the DM network is marked by basal and stimulus-evoked (such as by negative pictures) hyperactivity. The DM network remains hyperactivated during externally directed attention-demanding tasks (Sheline et al. 2009); hence, the disordered self-referential thought and obsessive ruminations. MDD patients also exhibit attention and processing bias that is associated with increased and sustained activity within the amygdala. The processing of reward and of emotional valence—two core symptoms of MDD—is related to both the PFC and the amygdala. For example, functional MRI studies involving reward-processing tasks showed reduced activity in the PFC and this is associated with anhedonia ratings (Keedwell et al. 2005). Depressives show exaggerated hemodynamic responses of the amygdala to sad words or sad faces, but blunted responses to masked happy faces (see Victor et al. 2010 for a review). Naturally, other brain areas are associated with the psychopathological processes observed in MDD. In particular, the nucleus accumbens (NAc) fails to respond to rewards in MDD patients, which is related to a decreased

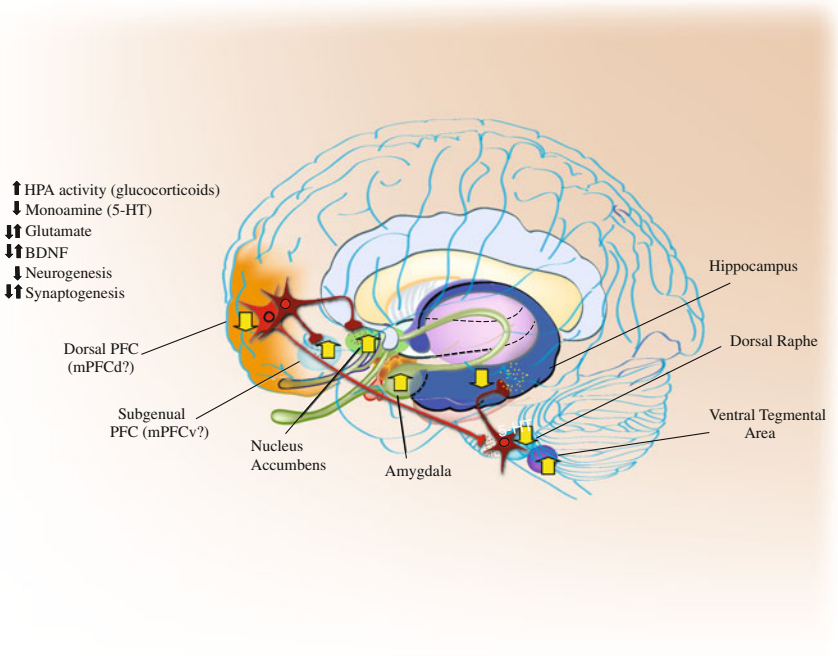

Fig. 1 The activity of a number of neuroanatomical nodes (as indicated) of the limbic neural network is differentially affected in major depressive disorder. The activity of the hippocampus and the prefrontal cortex (PFC), two of the major hubs within the network, is compromised. The dorsolateral PFC—thought to correspond to the rodent dorsomedial PFC (mPFCd)—is downregulated while the subgenual PFC—thought to correspond to the rodent ventromedial PFC—is upregulated. This disruption in the normal balance of nodal activity adversely impacts information flow through neurons, and is associated with a number of endophenotypes: HPA (glucocorticoid) activity, monoamine (serotonin) levels, glutamatergic activity, BDNF, hippocampal neurogenesis, and synaptogenesis (*arrow up* = increased, *arrow down* = decreased, *mixed arrows* = region-specific). *5-HT*, 5-hydroxytryptamine/serotonin; *BDNF*, brain-derived neurotrophic factor; *HPA*, hypothalamic–pituitary–adrenal axis

volume of this brain area (Epstein et al. 2006; Pizzagalli et al. 2009). The lateral habenula, a brain area involved in the modulation of the salience of aversive events, has been found to show structural abnormalities in MDD subjects (Ranft et al. 2010). Most of these abnormalities are improved by pharmacotherapeutic and psychotherapeutic interventions, and functionalities are altered in relation to remission and relapse. These observations have in recent years been enriched by research into deep brain stimulation (DBS) and repetitive transcranial magnetic stimulation (rTMS), in that it was made clearer how stimulation or inhibition of one region functionally relates to the activity of others, and how this impacts depressive symptoms (Mayberg et al. 2005). Figure 1 illustrates changes in the different structures of the depressive corticolimbic network.

4.2 The Network Hypothesis in Animal Models

The many limitations of currently used animal-based behavioral paradigms, which at best model individual components of depression constrains exhaustive testing of the mood-regulating (DM) network, particularly its response to genetic, epigenetic, pharmacological, and other environmental manipulations (for a comprehensive review of the validities of animal models of depression, see Nestler and Hyman 2010; Belzung and Lemoine 2011). Nevertheless, the neurosciences have gone a long way in understanding the effects of ADTs, stress, and of other presumed depressogenic stimuli on metabolic/physiological activity and signal propagation within limbic structures using modern electrophysiological and in vivo imaging techniques. More recently, optogenetic techniques have provided exciting prospects for future endeavors. Along with these developments, considerable efforts have been directed at examining the molecular, cellular, and neuroplastic events within major neuroanatomical hubs that are associated with documented changes in neurophysiological activity found in animal models of depression. These animal data, when combined with human postmortem studies, provide valuable insights into whether molecular and morphological correlates (at the neuronal and synaptic levels) of MDD underlie the remodeling of circuits/network and resultant perturbations of information flow within the network.

5 Depression, Antidepressants, and Neurogenesis

5.1 Neurogenesis in the Adult Mammalian Brain

In recent years, much interest in research on MDD and ADTs has focused on hippocampal neurogenesis. Indeed, it is now well established that in some brain areas of adult mammals, neural precursor cells can differentiate to all types of neural cells, including neurons, astrocytes, and oligodendrocytes (Kempermann and Gage 2000). The new neurons establish functional connections and exhibit electrophysiological activity. Although some evidence suggests that adult neurogenesis may also occur in other areas, including the hypothalamus, amygdala, neocortex, piriform cortex, cerebellum, substantia nigra, and striatum (Ponti et al. 2006; Gould 2007), new neurons have mainly been described in two regions: the subgranular zone of the hippocampus (SGZ) and the olfactory bulbs [to which new neurons migrate from cells that have proliferated within the subventricular zone (SVZ)]. It has thus been suggested that specific microenvironments of the SGZ and SVZ, termed as the neurogenic niche, may be permissive for the differentiation and integration of new neurons. Mitotic cells in both SGZ and SVZ are closely associated with the vasculature, suggesting that the blood vessels might release some factors stimulating adult neurogenesis (Alvarez-Buylla and Lim 2004; Palmer et al. 2000). These new neurons are generated from radial glia-like stem cells

(Type-1 cell) that produce intermediate precursor cells (Type-2a cell) that are amplified and acquire neural fate (Type-2b cell). They then differentiate into progenitor cells (Type-3 cell) maturating into granular neurons. The duration of this process is about 4–8 weeks, even if these cells continue to mature both physiologically and morphologically later on (Ge et al. 2007; Toni et al. 2008; Zhao et al. 2006). About half of newborn neurons die within 4 weeks after birth and this is related to the apoptotic gene Bax. It has been suggested that only those cells that were able to functionally integrate might survive. Different stages of the process leading to the generation of new hippocampal neurons, including proliferation, but also differentiation and survival are regulated by several factors including stress, aging, enrichment, physical exercise, neurotrophic factors, hormones, and neurotransmission. Interestingly, as mentioned above, most of these factors are also associated with the etiopathogeny of MDD or with ADT effects. More precisely, factors that negatively regulate hippocampal neurogenesis, such as stress, glucocorticoids or aging, or some comorbid diseases (for example, diabetes), are also associated with increased risks for MDD, while factors that stimulate hippocampal neurogenesis, such as physical exercise, neurotrophic factors, some neurotransmitters, and ADT therapy, seem to prevent MDD.

5.2 Factors Affecting Hippocampal Neurogenesis

Indeed, stress results in decreased cell proliferation within the SGZ and/or in hippocampal neurogenesis. This has been seen in different species (tree shrews: Gould et al.1997; monkeys: Gould et al. 1998; rodents: Mineur et al. 2007; Alonso et al. 2004) and with different types of stressors including repeated restraint stress (Pham et al. 2003), unpredictable chronic mild stress (Mineur et al. 2007; Alonso et al. 2004), or physical stressors (Malberg and Duman 2003; Pham et al. 2003; Vollmayr et al. 2003). Furthermore, this concerns all stages leading to the generation of new neurons, as stress reduction in hippocampal cell proliferation, as well as neuronal survival have been described (Czeh et al. 2001, 2002; Mirescu and Gould 2006; Oomen et al. 2007; Wong and Herbert 2004). However, this reduction also depends on the ability to cope with the stressful situation. For example, using the learned helplessness protocol, a widely used rodent model of MDD, Shors et al. (2007) reported that controllable stress (rats were trained to escape electric shock) caused reduction in SGZ cell proliferation of a smaller amplitude than uncontrollable stress (rats were given the same amount/intensity of shocks as in the first group, but in a yoked manner, with no possibility of escape). Other studies further showed that if coping with stress is possible, this even increases neurogenesis in rodents and monkeys (Lyons et al. 2010; Parihar et al. 2011). The above-mentioned effects of stress on neurogenesis have been suggested to be related to the fact that glucocorticoids induce a robust decrease in cell proliferation and differentiation into neurons, as well as of survival of new hippocampal neurons (Gould et al. 1992; Cameron and Gould 1994; Wong and

Herbert 2004; Montaron et al. 2003). However, things are more complex as effects of stress on neurogenesis do not fully parallel stress effects on glucocorticoid levels. For example, it has been recently shown that severe social stress elicits a long-lasting effect on the number of immature hippocampal neurons, which persisted even once corticosterone levels had normalized completely (Van Bokhoven et al. 2011). Others suggested that the stress-induced decrease of neurogenesis might rather be mediated by the proinflammatory cytokine interleukin-1 beta (IL-1β) receptor. Indeed, blockade of the IL-1 β receptor, IL-1RI, suppresses the stress-induced decrease of hippocampal neurogenesis (Koo and Duman 2008).

Aging, another factor associated with increased risk for MDD, also elicits decreased cell proliferation and/or neurogenesis in the hippocampus (Kuhn et al. 1996; Bondolfi et al. 2004; Rao et al. 2006; Heine et al. 2004). For example, hippocampal neurogenesis decreases linearly with age in macaques (Leuner et al. 2007).

Conversely, physical exercise has also been shown to elicit both beneficial effects in MDD (for a review see Knöchel et al. 2012) and increased hippocampal neurogenesis. Indeed, when rodents are given free access to a running wheel, they run as much as 3–8 km a night, and this promotes hippocampal neurogenesis. This effect can be seen as early as 24 h after the start of exercise, but the most convincing effects ensue after 3 days of exercise (van Praag et al. 1999; Kronenberg et al. 2003). This effect is predominantly related to stimulation of intermediate progenitor cells (Type 2a/b) and late progenitor cells (Type 3) (see Fabel and Kempermann 2008). Rearing of rodents in enriched environments can also relieve stress-induced depressive and anxious behaviors, and this effect is related to, or even requires neurogenesis (Veena et al. 2009; Schloesser et al. 2010).

Interestingly, monoaminergic neurotransmission has also been linked to hippocampal cell proliferation and/or neurogenesis. Indeed, serotonin level is positively correlated with hippocampal neurogenesis (Brezun and Daszuta 1999, 2000; Banasr et al. 2004; Lucas et al. 2007; Radley and Jacobs 2002) and stimulation of several serotonergic receptors ($5HT_{1A}$, $5HT_{2A}$, $5HT_{2C}$, and $5HT_4$ receptors) induces an increase of proliferation and, in some cases, of differentiation and survival of new hippocampal neurons (Brezun and Daszuta 2000; Banasr et al. 2004, 2006; Santarelli et al. 2003; Kulkarni et al. 2002; Lucas et al. 2007). Furthermore, hippocampal cell proliferation is decreased by noradrenergic depletion while noradrenergic stimulation has opposite effects (Kulkarni et al. 2002; Rizk et al. 2006). These noradrenergic effects on neurogenesis involve the α2-adrenoceptors. Indeed, the α2-adrenoceptor agonists clonidine and guanabenz both decrease neurogenesis through a selective effect on proliferation, (Yanpallewar et al. 2010), while α2-adrenoceptor antagonists such as dexefaroxan elicit the opposite effect (Rizk et al. 2006). These effects have been suggested to be mediated by α2-heteroceptors located on progenitor cells.

5.3 Antidepressants and Hippocampal Neurogenesis

Of course, all this evidence suggests that hippocampal neurogenesis may in some way relate to MDD, but the argumentation remains rather indirect. The observation that all available or putative ADT drugs promote hippocampal neurogenesis provides a much stronger argumentation on this relationship between MDD and young hippocampal neurons. Indeed, in the early 2000s, it was shown that monoamine-acting ADTs all produce an increase in the number of new cells or new neurons within the hippocampus (Manev et al. 2001). Even if this effect has not been universally observed (Cowen et al. 2008; Hanson et al. 2011), it led to the general idea that therapeutic ADT action might relate to hippocampal neurogenesis. This became even more convincing when it was observed that the ADT effects on hippocampal neurogenesis could be seen after chronic ADT treatment, but not after acute injections. This is in fact easy to explain, as the differentiation of the hippocampal progenitors into mature granule neurons also takes several weeks (Encinas et al. 2006; Kempermann et al. 2004). In any case, this observation indicates that the action of monoaminergic ADTs on neurogenesis is correlated with the onset of the therapeutic effects of these compounds. The idea that the therapeutic effects of ADT drugs may in some way involve hippocampal neurogenesis received even further support from the observation that similar effects could be found with putative ADTs or ADT-like compounds acting via other, nonmonoaminergic pathways including glutamatergic agents (Yoshimizu and Chaki 2004), endocannabinoid ligands (Jiang et al. 2005), tianeptine (Czeh et al. 2001; McEwen et al. 2002), compounds acting on the stress axis such as CRF1 or vasopressin V1b receptor antagonists (Alonso et al. 2004) or a melanin-concentrating hormone (MCH) antagonist (David et al. 2007). Furthermore, the mood stabilizers lithium and valproate that relieve bipolar depression also markedly enhance both proliferation and survival of new hippocampal neurons (Chen et al. 2000; Hao et al. 2004; Silva et al. 2008; Hanson et al. 2011). In some cases, for example with SSRIs, proneurogenic effects were observed in normal rodents (Malberg et al. 2000) while in other cases (for example with the CRF1 antagonist), ADTs restored instead the stress-induced decrease in neurogenesis (Alonso et al. 2004). The observation that nonpharmacological treatments able to relieve MDD elicited similar effects further contributed to the tenability of the hypothesis that therapeutic effects are mediated via neurogenesis more convincing. Indeed, electroconvulsive therapy (Malberg et al. 2000), as well as VNS both increase hippocampal neurogenesis (Revesz et al. 2008). However, the picture is not monolithic. For example, rTMS, a treatment having ADT properties, only partly abolishes the stress-induced decrease of cell proliferation, while it suppresses the survival rate of proliferating cells (Czeh et al. 2002). The idea about the involvement of young hippocampal neurons in ADT action also received support

from clinical evidence, as proneurogenic effects of ADTs could also be observed in humans (Boldrini et al. 2009), although contradictory findings have also been reported (Reif et al. 2006).

Do chronic ADTs act on one particular stage of the generation of new hippocampal neurons? Some studies investigated this issue, mainly focusing on the effects of the SSRI fluoxetine. First, in 2006, Encinas and coworkers showed that fluoxetine specifically targeted the proliferation of neural progenitor cells and not the number of neuroblasts or of immature neurons. However, more recently it was shown that fluoxetine decreased the number of immature neurons and increased the number of mature ones, suggesting that it accelerated the maturation of new immature hippocampal neurons (Wang et al. 2008). Regarding survival, a study showed that 2 weeks of fluoxetine did not affect this process (Malberg et al. 2000), but it was shown later that 4 weeks of fluoxetine was effective in stimulating cell survival (Nakagawa et al. 2002). Moreover, fluoxetine also acts on the functional properties of the new hippocampal cells as it stimulates neurogenesis-dependent long-term potentiation (LTP) in the dentate gyrus (DG) (Wang et al. 2008).

Most of the above-mentioned studies relating ADT effects with hippocampal neurogenesis are correlative. Causal relationship was investigated using different tools to suppress new hippocampal neurons including focal hippocampal irradiation, systemic administration of methylaxoxymethanol (MAM: an anti-mitotic drug), or using transgenic mice (hGFAPtk mice: administration of Ganciclovir to mice carrying the transgene results in the death of dividing neural stem cells (GFAP cells) expressing the herpes simplex virus expressing thymidine kinase (HSV-TK)). The first of these studies used hippocampal focal X-ray irradiation to suppress proliferating cells in the DG. This ablated newborn hippocampal neurons, as well as the behavioral effects of chronic fluoxetine in several situations, including the novelty suppression of feeding test, the splash test, the coat state, and the motivation for a reward (Santarelli et al. 2003; Surget et al. 2011). Similar effects were observed using the TCA imipramine (Surget et al. 2008), which were also confirmed by several other studies investigating the causal role of neurogenesis in the effects of monoaminergic ADTs using focal hippocampal irradiation (Wang et al. 2008; Airan et al. 2007). However, these data have not always been replicated thereon, and several explanations can be provided for this discrepancy: (A) The participation of hippocampal neurogenesis in ADT effects is strain-dependent, as it can be observed in some strains and not in others (Holick et al. 2008). (B) It might depend on the phenotype tested. Indeed, the effects of monoaminergic drugs are always suppressed by irradiation-induced ablation of neurogenesis in the novelty-suppressed feeding test (Santarelli et al. 2003; Wang et al. 2008; David et al. 2009), on the coat state (Santarelli et al. 2003; Surget et al. 2008, 2011), in the splash test (Santarelli et al. 2003; Surget et al. 2008) and in tests for anhedonia (Perera et al. 2011; Surget et al. 2011), while neurogenesis does not seem to be required for the effect of fluoxetine in the open field test and in the forced swim test (David et al. 2009). One may propose that these later tests have

little relevance for ADT-like compounds, and thus conclude that the requirement for neurogenesis in the ADT effect only concerns phenotypes relevant for chronic ADT action. (C) It might rely on the methodology used to ablate neurogenesis. For example, one study used a 2-week administration of the anti-mitotic MAM to prevent the formation of new neurons (Bessa et al. 2009). MAM was applied during the last 2 weeks of a stress protocol, together with the ADT therapy. In this study, the effects of fluoxetine and imipramine were still present after suppression of new cells, but this was probably due to the fact that blocking mitosis during 2 weeks before testing only suppressed progenitor and neuroblasts, while young neurons aged more than 2 weeks were still present. Probably the ablated cells were too young to have a functional role. To test for this, a study should be undertaken in which the effects of ADT therapy might be tested 4 weeks after MAM administration. (D) It might depend upon the ADT treatment used. Indeed, while neurogenesis seems important for the action of monoaminergic compounds, as well as for cannabinoid ligands (Jiang et al. 2005), the studies investigating the causal participation of neurogenesis in the effects of HPA-acting agents such as CRF1 or V1b antagonists never found that new neurons were required for these effects (Surget et al. 2008, 2011; Bessa et al. 2009). This similarly applies to the MCH1 antagonist (David et al. 2007), as well as for some nonpharmacological therapies. For example, neurogenesis seems required for the ADT-like effects of intermittent hypoxia (Zhu et al. 2010), but does not seem necessary for the ADT-like effects of running (Fuss et al. 2010). On the other hand, divergent results were found concerning environmental enrichment, some finding neurogenesis necessary (Schloesser et al. 2010) and others not (Meshi et al. 2006). To summarize, if young neurons aged 4–6 weeks are ablated, ADT-like effects of monoaminergic compounds are somewhat always suppressed in tests sensitive to chronic ADT treatment (there is just one exception in the literature).

However, even if neurogenesis is causally involved in the ADT-like action of some pharmacological treatments in achieving remission, this does not elucidate which physiological or psychological process is underlying these effects. To answer this question, it is necessary to understand the functions of adult new hippocampal neurons. The hippocampus is involved in many functions, including learning and memory, spatial navigation, pattern separation, anxious behavior, and HPA axis regulation. Hippocampal neurogenesis has been shown to be involved in some of these functions. Indeed, pattern separation is altered after the depletion of newly generated neurons (Tronel et al. 2012; Clelland et al. 2009), while the opposite is observed after an increase in the number of new neurons (Sahay et al. 2011). Furthermore, decreased neurogenesis deteriorates hippocampal-dependent learning and memory (see Koehl and Abrous 2011 for a review). However, it is unlikely that these functions are sufficient to explain the ability of ADTs to achieve remission. Indeed, to our knowledge no study has investigated the capacity of ADT drugs to improve pattern separation, and even if a deficit in pattern separation could induce an overgeneralization to negative cues, it should in the same manner

induce an overgeneralization to positive cues. If the first of these processes can participate in precipitating a depressive episode, the second one would certainly have the opposite action! The same reasoning applies to the involvement of hippocampal neurogenesis in learning and memory: Even if MDD patients may show a deficit in learning and memory, it is difficult to understand how improving this aspect would achieve remission. Others observed that suppression of neurogenesis elicits increased anxious behavior in bioassays screening state anxiety (Revest et al. 2009). It is known that anxiety disorders are comorbid with MDD, so one can hypothesize that ADT drugs would in fact inhibit the anxiety component of ADT treatment, facilitating recovery. However, this is not really plausible, as ablation of hippocampal neurogenesis does not suppress the effects of the anxiolytic properties of fluoxetine in the open field (David et al. 2009) or the anxiolytic effects of running in the open field and in the light/dark tests (Fuss et al. 2010). Hence, another function of the hippocampus in general and of hippocampal neurogenesis in particular should explain the capacity of ADT drugs to elicit remission via their action on neurogenesis. One good candidate for this is the involvement of the hippocampus in mediating negative feedback inhibition of the HPA axis (Herman and Mueller 2006). We have mentioned earlier that MDD patients exhibit a deficit of this regulation and that reversal of this defect seems necessary for the therapeutic effects of ADTs (see above). Indeed, it is possible that hippocampal neurogenesis might play an important role in this feedback. A recent study has shown that suppression of neurogenesis led to increased release of glucocorticoids following exposure to a mild stressor, suggesting impairment in negative feedback inhibition (Schloesser et al. 2009). It has also been shown that fluoxetine recruits new neurons to induce therapeutic effects, and that this is suppressed after irradiation (Surget et al. 2011). It is therefore conceivable that monoaminergic drugs achieve therapeutic efficacy by stimulating neurogenesis and restoring a proper hippocampal function, notably the hippocampus-driven inhibition of the HPA axis.

5.4 A Causal Link Between Depression and Hippocampal Neurogenesis?

Finally, does a decrease in neurogenesis cause depression-like behaviors? Here, divergent experimental evidence exists. Indeed, most studies did not show any effect of ablation of neurogenesis on depression-related behaviors. For example, using focal irradiation to block neurogenesis, no effects were found in the suppression of feeding test (Santarelli et al. 2003; Meshi et al. 2006; Surget et al. 2008; Wang et al. 2008; David et al. 2009; Zhu et al. 2010), in the forced swim test (Airan et al. 2007; Holick et al. 2008; Revest et al. 2009; Zhu et al. 2010; David et al. 2009), on the coat state (Santarelli et al. 2003; Surget et al. 2008, 2011; Zhu et al. 2010), in the splash test (Santarelli et al. 2003; Surget et al. 2008), on sucrose

consumption or preference (Noonan et al. 2010) or on consumption of chocolate cookie (Surget et al. 2011). Thus, focal irradiation—the sole ablation strategy specifically disrupting hippocampal neurogenesis—never induces a depressive-like phenotype, and this applies to several test situations, as well as to intact/naive or stressed rodents. If ablation of neural progenitor cells and of neuroblasts is achieved via MAM, effects can be observed in the novelty-suppressed feeding tests, but neither in the forced swim test nor on sucrose consumption (Bessa et al. 2009; Jayatissa et al. 2010). As mentioned above, this strategy not only suppresses new hippocampal cells as well as new olfactory bulb cells, but it also suppresses mitotic cells, and not young neurons. Finally, if neurogenesis is ablated using genetic manipulations, effects depend upon the mutant used and on the experimental situation. Indeed, if the genetic manipulation targets Nestin-expressing cells, no effects could be observed in the tail suspension test (Revest et al. 2009; Singer et al. 2009) or in the novelty-suppressed feeding test. Conversely, if the genetic manipulation targets GFAP-expressing cells, the picture appears rather complex, as effects depend upon the test and the stress level of the mice. Indeed, at a basal stress level, these mice show no alteration in the novelty-suppressed feeding test while they display decreased sucrose preference and increased latency to immobility in the forced swim test (Snyder et al. 2011). After restraint stress, the effect on the forced swim test is no longer present, while mice exhibit decreased sucrose preference and increased latency in the novelty-induced suppression of feeding test (Snyder et al. 2011). However, this study is difficult to interpret as neurogenesis is decreased in both the hippocampus and the olfactory bulbs, and some peripheral side effects could have induced nonspecific behavioral alterations (Wei et al. 2011; Bush et al. 1998). In conclusion, most studies did not show a causal participation of young hippocampal neurons in triggering depression-like behaviors. The sparse evidence that suggests otherwise could mean that at least in part impaired hippocampal neurogenesis confers vulnerability to depression. It is also possible that this could occur indirectly given that different regions of the hippocampus along its septotemporal axis could subserve different neurogenesis-dependent functions (Bannerman et al. 2004; Fanselow and Dong 2010; van Strien et al. 2009). While its ventral aspect appears to be involved in the modulation of emotional behavior (Bannerman et al. 2004; Fanselow and Dong 2010; Hobin et al. 2006; Kjelstrup et al. 2002; Pentkowski et al. 2006), its dorsal aspect mainly exerts learning and memory functions (Bannerman et al. 2004). This functional dissociation could also be attributed to differences in the afferent and efferent inputs along the septotemporal axis, with the ventral component receiving denser serotonergic innervation (Bjarkam et al. 2003; Gage and Thompson 1980; Wilson and Molliver 1991), and sending rich projections to the PVN, amygdala, basal nucleus of the stria terminalis, and the medial PFC (Bannerman et al. 2004; Fanselow and Dong 2010; van Strien et al. 2009).

6 Depression, Antidepressants, and Synaptogenesis

6.1 Synaptogenesis and the Developing Brain

Whereas the relationship between hippocampal neurogenesis and MDD pathophysiology warrants further clarification, another key neural substrate that has been implicated in both MDD and in the effects of ADTs are subcellular dynamics related to the formation and maintenance of synapses (synaptogenesis). Synaptogenesis begins with the synthesis of receptors, adhesion molecules, and scaffolding proteins at the level of the soma, the products of which are subsequently sorted and then trafficked down to neurites (axons and dendrites). The clustering of these proteins at the presynaptic compartment [for example, synaptophysin and soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) complex], but especially profound at the postsynaptic density (PSD) (such as PSD-95, beta-neurexin, neuroligin I, metabotropic and ionotropic glutamate receptors, actin, etc.) galvanizes the synaptic connection at initial sites of contact, and facilitates axon and dendrite convergence (Bresler et al. 2001, 2004; Friedman et al. 2000; Garner et al. 2002; Shapira et al. 2003; Ziv and Garner 2001, 2004; Washbourne et al. 2002). This process generally requires presynaptic and postsynaptic mechanisms, eventually influences functional plasticity [LTP or long-term depression (LTD)], and renders profound mutability to the very basic units of communication within a network. Even if spinogenesis does not occur, changes in spine morphology are sufficient to modify synaptic strength, without necessitating spine turnover (Huttenlocher 1990; Bhatt et al. 2009). As such, impairments in synaptogenesis, particularly in the maintenance of formed synapses, may well underlie the reorganization and disarray in neural networks essential to stress adaptation, such as that seen to increase vulnerability to MDD. However, the question of whether synapse elimination or constrained synaptogenesis is necessarily bad is more complex. Fluctuations in synaptogenesis are but natural occurrences during early ontogenetic development, where both enrichment and regression of synaptic contacts are essential to the eventual stabilization of neural networks and normal brain function. During early postnatal life, cortical synaptogenesis rapidly peaks, but is inevitably from thereafter and through adolescence followed by dramatic elimination of formed synapses (Huttenlocher 1990). The spatiotemporal progression of synaptogenesis and synaptic elimination indicates a collect-and-select strategy of stabilizing neural networks. In the adult brain, where synaptogenesis is fairly limited, some spines are constantly emerging and retracting, but the majority are persistently stable for months and years (Grutzendler et al. 2002; Bhatt et al. 2009). The spatial (location in the brain) and temporal (onset and termination) characteristics of adult synaptogenesis, spinogenesis, and spine elimination, and the relative influences of experience and environmental factors on them, are gradually being unraveled (Bhatt et al. 2009). With the utilization of novel techniques, including two-photon microscopy (real-time imaging of several cortical layers of fluorescently labeled synapses in vivo),

in conjunction with ex vivo histological and molecular imaging techniques, we are beginning to explore these questions within the context of MDD and ADT action.

6.2 *Depressogenic Stimuli Compromise the Structure of the Synapse*

The notion that structural synaptic changes contribute to MDD pathophysiology is supported by observations of modified expression levels of synapse-associated proteins. Animals submitted to chronic stress with homotypic (such as repeated restraint) or variable (unpredictable) stimuli develop depression-like behaviors, such as anhedonia in the sucrose preference test, behavioral despair in the forced swim test or tail suspension test, enhanced anxiety in the novelty-suppressed feeding test, and increases in plasma corticosterone levels (Bachis et al. 2008; Nestler and Hyman 2010). Accompanying these are reductions in the expression levels of the postsynaptic density protein 95 (PSD-95), which associates with receptors and cytoskeletal elements and facilitates excitatory presynaptic and postsynaptic maturation and enhancement in dendritic size and density; reductions in synapsin I, a phosphoprotein on synaptic vesicular membranes, regulating axonogenesis and synaptogenesis; reductions, in the AMPA-type glutamate receptor subunit 1 (GluR1), whose insertion is required for spine enlargement; and reductions in synaptophysin, an activity-dependent regulator of synapse formation, and a marker for synapse quantity (Li et al. 2010; Elizalde et al. 2010; Muller et al. 2011). There are also reports that these synaptic elements may already be affected at the genetic and transcriptional levels (Muller et al. 2011; Duric et al. 2012). Although these changes have been found in both the hippocampus and PFC (Li et al. 2010; Elizalde et al. 2010; Muller et al. 2011), some evidence suggests that the PFC is more sensitive to this effect of stress (Muller et al. 2011), indicating that structural impairment in the PFC may come as an initial phase that could gate more far-reaching disturbances in neural transmission and plasticity. In addition to these synaptic proteins directly participating in synaptogenesis, several other ones involved in presynaptic vesicular docking, fusion, and exocytosis (neuronal transmission) are also impacted by stress in a complex manner. A number of these essentially constitute or interact with the SNARE complex, and include among others syntaxin 1A, synaptosomal-associated protein 25 (SNAP-25), vesicle-associated membrane proteins (VAMP 1 and 2), SNARE-associated protein implicated in synaptic transmission (snapin), synaphins (complexins I and II), and the calcium sensor synaptotagmin. In the PFC, chronic restraint increases the transcripts of many of these presynaptic proteins, except those of SNAP-25 that has been shown to be downregulated (Muller et al. 2011); while rats inbred for congenital learned helplessness, a genetic model of MDD, have been found with decreased mRNA levels of synaphins in the hippocampus and cortical areas (Zink et al. 2007). Many of these abnormalities in synaptic protein expression mirror

those found in MDD patients, analyzed postmortem. Reductions in the expression levels of PSD-95 and of dihydropyrimidinase-related protein 2 (DRP-2, participates in axonal growth and guidance, neural differentiation, and microtubule assembly), along with those of the NMDA receptor (NMDAR) NR2A and NR2B subunits and of metabotropic glutamate receptor (mGluR) 5, whose activation are as well implicated in synaptogenesis, were detected in the PFC of MDD patients (Deschwanden et al. 2011; Feyissa et al. 2010; Johnston-Wilson et al. 2000; Jernigan et al. 2011). The presynaptic docking/fusion-associated protein synaphin has also been shown to be affected in this region (Sawada et al. 2002), as well as in the anterior cingulate (Eastwood and Harrison 2000, 2001). In the hippocampus, MDD has been found to be associated with significant dysregulation of genes involved in the regulation and maintenance of synaptic function and structure, along with those involved in serotonergic receptor functions. These include those of the postsynaptic scaffolding protein disks' large homolog 2 (DLG2, regulates protein clustering at the postsynaptic density during synaptogenesis), of the microtubule-associated protein 1A (MAP1A, modulates microtubule dynamics importantly involved in neuronal morphogenesis and neurogenesis), as well as those of GLUR1 and GLUR3 AMPA receptor (AMPAR) subunits (Duric et al. 2012). The direction of alterations in the expression of synaptogenesis-related proteins in the hippocampus needs to be further explored. Nevertheless, there have been reports of abnormalities in presynaptic docking/fusion-associated proteins (such as SNAP-25 and synaphins) in the hippocampus (Fatemi et al. 2001; Honer et al. 2002; Eastwood and Harrison 2000, 2001; Zink et al., 2005; Reines et al. 2008) of MDD patients, along with abnormalities in synaphin/complexin expression in the anterior cingulate (Eastwood and Harrison 2000, 2001) and PFC (Sawada et al. 2002).

From the structural standpoint, the effects of depressogenic stimuli on synapse-associated proteins may well indicate insults to neurite (axons and dendritic spine) morphology, malformation, and synaptic remodeling consequential to neuronal atrophy, and/or aberrations in spinogenesis and synaptogenesis. In fact, together with changes in presynaptic protein expression, cortical elevations in apoptosis-related proteins, such as those of caspases, have been found in animals exposed to chronic unpredictable stress (Bachis et al. 2008), indicating synaptic deterioration and neuronal atrophy. Indeed, two-photon laser imaging and ex vivo morphological analyses of prelabeled pyramidal neurons in almost all layers of the prelimbic PFC revealed chronic stress-induced reductions in spine density at both distal and proximal segments of the apical tuft in both rats (Brown et al. 2005; Radley et al. 2006; Li et al. 2011a) and mice (Hill et al. 2011). Particularly affected profoundly are mature mushroom-like (large diameter, short length) spines, indicating considerable deterioration of mature spines, and compromising synaptic contacts (Li et al. 2011a). This effect of chronic unpredictable stress is recapitulated in other animal models, such as in cannabinoid CB1 knock-out mice (Hill et al. 2011). A similar scenario was found in the hippocampus, where prolonged cell loss, synaptic degeneration, and shrinkage of dendritic arborizations in the CA3 region ensue after chronic unpredictable stress, chronic restraint, or chronic

corticosterone administration (McEwen 1999, 2001; Magarinos et al. 1997; Joels et al. 2004). Stereological analyses in the Flinders sensitive line of rats also showed decreases in the volume and in the number of hippocampal neurons and spine synapses, when compared to the Flinders resistant ("normal") rats (Chen et al. 2010). Flinders sensitive rats display a hypersensitivity to cholinergic (muscarinic) agonists and a genetic susceptibility to depression-like behavior, particularly manifested in response to the acetylcholinesterase diisopropyl fluorophosphates. These observations made from animal models are arguably generalizable to the human population, as postmortem analyses have indeed determined an association between mood disorder and structural abnormalities and spine density in the cerebral cortex, hippocampus (subiculum), and anterior cingulate (Rosoklija et al. 2000; Eastwood and Harrison 2000, 2001). The role of abnormal synapse-associated protein expression and subsequent synaptic structural changes in MDD and in animal models is much appreciated by the fact that these changes are generally reversible by thymoleptics and ADTs, including physical exercise and exposure to enriched environments (Rapp et al. 2004; Zink et al. 2005; Reines et al. 2008; Li et al. 2010; Czeh et al. 2001; Sheline et al. 2003; Radley et al. 2004; Vaynmana et al. 2006; O'Leary et al. 2009; Chen et al. 2010; Duric et al. 2012). It is therefore plausible that the decrease in hippocampal and PFC volume in MDD and their reported increase after chronic ADT treatment are brought about by retardation and enhancement, respectively, of synaptogenesis, in addition to possible changes in hippocampal neurogenesis (Boldrini et al. 2009; Price and Drevets 2010).

6.3 Role for Activity-Dependent Neurotrophic Signaling

As far as evidence from preclinical and clinical data suggest, structural synaptic changes associated with stress and MDD may be nuanced by adverse consequences on neurotrophic factors [brain-derived neurotrophic factor (BDNF), vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), VGF, insulin-like growth factor (IGF), nerve growth factor (NGF), and neurotrophin-3] (Nibuya et al. 1995; Duman et al. 1997; Nestler et al. 2002; Dias et al. 2003; Duman and Monteggia 2006; Tanis and Duman 2007; Duman and Voleti 2012). MDD adversely influences the activity of a number of signaling proteins associated with neurotrophic signaling, via numerous signal transduction cascades, an effect opposite to those induced by ADTs. In fact, the augmented levels of serotonin and norepinephrine elicited by chronic administration of conventional ADTs are known to stimulate attendant receptors that are linked to the activation of these intracellular mechanisms. These ADTs ultimately influence nuclear changes, including activation of transcription factors such as the cyclic adenosine monophosphate regulating element binding protein (CREB). Interestingly, chronic, but not acute administration of conventional ADTs of several classes have been found to increase the expression of CREB in the hippocampus (Nibuya et al. 1996), while

CREB is decreased in postmortem studies of depressed suicide victims and increased by premortem ADT treatment (Pittenger and Duman 2008). CREB in turn stimulates transcription of some other genes of interest, such as one coding for BDNF. Since the formulation of the neurotrophic hypothesis of MDD (Duman et al. 1997) more than a decade ago, considerable attention has been directed to BDNF, and its impaired production (Duman and Monteggia 2006). Indeed, BDNF levels are decreased in the brains of suicide victims, but increased by premortem ADT treatment (Karege et al. 2005; Pittenger and Duman, 2008), suggesting a strong link (arguably causal) between BDNF levels and disease states or therapeutic effects. Some studies did not fully conform to this notion. In particular, conclusions drawn from BDNF heterozygous, conditional, region-specific knockout/knockdown, and by way of the Cre/loxP recombination system, that induce full or forebrain-specific impairment of either BDNF or of its receptor, tropomysin-related kinase B (TrkB), only showed that the loss of BDNF at best undermines the efficacy of ADT drugs (Saarelainen et al. 2003; Monteggia et al. 2004; Deltheil et al. 2008; Ibarguen-Vargas et al. 2009; Autry and Monteggia 2012). Nevertheless, the observation that chronic unpredictable stress in animals leads to reduction in BDNF levels is consistent with findings that levels of BDNF and its cognate receptor are decreased in hippocampal postmortem tissue and in the serum of MDD patients (Castrén et al. 2007; Castrén and Rantamäki 2010a, b; Thompson Ray et al. 2011; Autry and Monteggia 2012). These effects on BDNF levels could be attributed to adverse influences on the activity of transcription factors, particularly CREB, via a myriad of signal transduction cascades, which are also integral to the function of other neurotrophic factors, as mentioned earlier. These pathways include the 3',5'-cyclic adenosine monophosphate (cAMP) second messenger system, the extracellular signal-regulated kinase/mitogen-activated protein kinase (ERK-MAPK), the phosphoinositol, the glycogen synthase kinase 3 (GSK-3), and the mammalian target of rapamycin (mTOR) pathways, which have all been identified to be involved in mood regulation and stabilization, and converge upon the expression of other plasticity-related genes that promote synaptogenesis, growth, neuroprotection, resiliency, and cell survival (Tanis and Duman 2007; Tanis et al. 2007; Duman and Voleti 2012; Fricker et al. 2005). The transcription of *BDNF* and consequential synaptogenesis may preferentially depend upon neural activity (Katz and Shatz 1996; Yin et al. 2002; Greer and Greenberg 2008). In other words, these require the influx of calcium mainly through L-type voltage-sensitive calcium channels (Hardingham et al. 1997; Papadia et al. 2005; Greer and Greenberg 2008) and the stimulation of glutamate receptors (NMDAR, AMPAR, mGluR1, and mGluR8), which in turn are regulated by protein synthesis and receptor trafficking (Roche et al. 2001; Prybylowski et al. 2005; Elias et al. 2006). The activity-dependent mechanism of BDNF activation may serve as a major trajectory recruited by ADTs in bequeathing structural synaptic changes. Indeed, chronic ADT administration modifies monoamine and excitatory neurotransmission and facilitates LTP, which also requires the stimulation of these glutamate receptors via high-frequency presynaptic depolarizations. Conversely, stress-induced structural regression is associated with functional impairments in

LTP (Karst and Joels 2003; Alfarez et al. 2002, 2003; Maroun and Ritcher-Levine 2003). The late phase of LTP (L-LTP) harnesses transcriptional and translational machineries necessary for structural synaptic modification, and this has been shown to depend upon BDNF (Messaoudi et al. 2002). Among the 8 *BDNF* promoters that altogether transcribe 18 mRNAs, promoters I and IV are most reactive to neural activity. It is hypothesized that only those mRNAs produced by these promoters tagged by their unique 3' and 5' untranslated regions (UTRs) are trafficked , captured and bound by TrkB, activated within the stimulated dendrites, hence, selectively participate in the strengthening of those synapses (Greer and Greenberg 2008; Liu et al. 2011). Mice that lack these tags, particularly transcripts lacking a long 3' UTR and those with disruption in their assigned promoters (such as promoter IV mutants) exhibit defective dendritic spine morphogenesis and synaptic plasticity (An et al. 2008), and produce depression-like behavior (Sakata et al. 2010). It is worth mentioning that the precursory pro-BDNF and its cognate receptor p75 exert effects opposite those of mature BDNF, that is, suppressive to dendritic spine growth. Pro-BDNF has to be converted into mature BDNF by the tPA-plasmin system before TrkB could be activated. Therefore, the balance between the anabolic function of mature BDNF-TrkB signaling and the catabolic function of pro-BDNF-p75 signaling (Woo et al. 2005; Deppmann et al. 2008) determines the direction and extent of synaptic strengthening through neurite modification (Martinowich et al. 2007). In addition, genetic vulnerabilities to BDNF, particularly its dendritic translation and release have also been accounted to contribute to structural synaptic changes. For instance, knock-in mice with the common human BDNF Val66Met (G1916A) polymorphism shows impaired trafficking of BDNF mRNA to dendrites by disrupting its interaction with RNA-binding protein translin (Chiaruttini et al. 2009). The Val/Met and Met/Met genotypes exhibit constitutive atrophy of distal apical dendrites, decreased spine density and diameter, and reductions in apically targeted excitatory postsynaptic currents in layer V pyramidal cells of the PFC, indicating impaired synaptic formation and maturation (Liu et al. 2011).

Within the context of a network assembly, the activity dependence of BDNF production and associated neuroplasticity possess a selective advantage. Established connections that subsequently become more electrochemically active than the rest or are contiguous with active ones, are spared from eventual pruning in the developing brain (Katz and Shatz 1996), and are strengthened in the adult brain (Greer and Greenberg 2008; Martinowich et al. 2007). Needless to say, this makes it conducive for the integration of new connections or for the replacement of aberrant ones within functional networks, following ADT treatment. However, the role of neural stimulation, particularly of glutamatergic excitatory transmission along microcircuits, is complex. Although as discussed above, postsynaptic excitation is necessary for synapse formation and growth, the pattern of AMPAR, NMDAR, and mGluR activation, and calcium channel phosphorylation, may determine whether synaptic assemblies are structurally and functionally reinforced. For one, calcium conductance through L-type voltage-sensitive calcium channels, as compared to other calcium and glutamate ion channels, better favors

gene expression responses, such as those of BDNF (Greer and Greenberg 2008). Secondly, phasic activation of synaptic NMDARs and of chronic activation of extrasynaptic NMDARs (located in the periphery of the PSD or adjacent to glia-like processes) elicits contrasting consequences. The former more specifically induces BDNF production, LTP, and anabolic/pro-growth signaling, while the latter induces synaptic and neuronal deterioration (Hardingham and Bading 2010). The pattern, intensity, and extent of glutamate mobilization and signaling could also contribute to the balance of activation of these synaptic and extrasynaptic NMDARs. In the hippocampus, the PFC and the amygdala, the effects of basal and evoked glutamate efflux on synaptic function and plasticity appear to assume a biphasic profile. Glutamatergic hyperactivity—as for example by heightened or prolonged stress, HPA overactivation or glucocorticoid administration (Lowy et al. 1995; Bagley and Moghaddam 1997; Reznikov et al. 2007; Musazzi et al. 2010; Liston and Gan 2011)—leads to disarrayed synaptic plasticity and disturbances in LTP (Maroun and Ritcher-Levine 2003; de Kloet et al. 2005; Popoli et al. 2011). This may occur when excessive glutamate results in increased or prolonged activation of extrasynaptic NMDARs (Hardingham and Bading 2010). The loss of glia in the depressive PFC, which has also been recently established empirically (Rajkowska and Miguel-Hidalgo 2007), could also exacerbate this occurrence by the insufficiency of glutamate clearance and reuptake through glial transporter molecules, although glia-secreted factors also facilitate by themselves synaptogenesis, synaptic maturation, and spine remodeling (Bolton and Eroglu 2009; Eroglu and Barres 2010). In addition to these, high glucocorticoid level may itself suppress BDNF activity or associated signaling pathways (such as MAPK/ERK) (Kumamaru et al. 2011). Altogether, these effects on neural activity and glutamatergic transmission contribute to the capacity of stress and ADTs to regulate neurotrophic factors (BDNF), consequently impacting the growth and maintenance of synapses (Kononen et al. 1994; Smith et al. 1995; Givalois et al. 2001).

6.4 Rapid Synaptogenesis Mediates the Fast Antidepressant Onset of Glutamate-Acting Agents

We have earlier discussed that chronic treatment with ADTs enhance hippocampal neurogenesis. We also presented a growing body of data indicating that ADTs likewise promote synaptogenesis in the hippocampus and the PFC. Both neurogenic and synaptogenic processes are linked with or are under the control of BDNF (Vaynmana et al. 2006; Calabrese et al. 2007; O'Leary et al. 2009; Castrén and Rantamäki 2010a, b). As evidence for the role of hippocampal neurogenesis is replete, the question of whether hippocampal and/or PFC synaptogenesis is essential, or at the very least, contributory to therapeutic ADT effects (and to MDD pathophysiology), is inevitably encountered. Interestingly, recent work on novel investigational ADT agents that elicit behavioral responses with relatively faster

onset or under experimental conditions precluding neurogenic influences has provided exciting insights. One such area investigates glutamate-acting, putative ADTs, such as NMDAR antagonists, ampakines, and mGluR antagonists. Remarkably, unlike slow-acting conventional ADTs, the NMDAR antagonist ketamine has been shown to robustly improve depression ratings in 70 % of patients, as well as effectively distil suicidality, just a few days (72 h) or even within hours after a single intravenous, low/subanesthetic-dose infusion, an effect that could last up to a week (Berman et al. 2000; Zarate et al. 2006; Zarate et al. 2012). This rapid and sustained ADT effect was reliably reproduced in animal models, including the forced swim test, tail suspension test, learned helplessness, novelty-suppressed feeding test, and sucrose preference test following chronic unpredictable stress (Li et al. 2010, 2011a; Autry et al. 2011), and was similarly elicited by NMDAR subunit NR2B antagonists (Li et al. 2010; Zarate et al. 2010), and possibly in slightly varying degrees by cognate compounds, such as MK-801, CPP, PCP, AP5, and memantine (Autry et al. 2011; Zarate et al. 2010). Subsequent studies have determined that this rapid ADT onset is forged by an immediate increase in synaptogenesis (marked by an increase in synapse-associated proteins, such as PSD-95, GluR1, and synapsin 1) detected in the PFC, an effect that ensued within a few hours after treatment and sustained for a week (Li et al. 2010, 2011a; Duman and Voleti 2012). In prelimbic PFC layer V pyramidal neurons, this was indicated by a significant increase in the number of dendritic spines, along with an enhancement in the amplitude and frequency of serotonin and hypocretin-induced excitatory postsynaptic currents (Li et al. 2010). Remarkably, while these synapse-related changes in the PFC were impaired by chronic unpredictable stress, they were nevertheless reversed by acute ketamine administration (Li et al. 2011a). Ketamine-induced synaptogenic and antidepressant effects were found to be linked to downstream action on translation-related kinases, particularly to the phosphorylation of the p70 ribosomal S6 kinase (p70S6 K), and repression of the eukaryotic initiation factor 4E (eIF4E)-binding protein (4E-BP) (Li et al. 2010) and of the eukaryotic elongation factor 2 kinase (eEF2 kinase or CAMKIII, also de-phosphorylated/inhibited by p70S6 K) (Autry et al. 2011). The action on these effectors cascade into the activation of their respective targets, the ribosomal subunit protein S6, eIF4E and eEF2, events leading to increased rates of the initiation and elongation phases of protein synthesis. These, proposed by Duman and colleagues (Li et al. 2010, 2011a), are accomplished by a transient activation of mTOR in the PFC, a ubiquitous, large (2549 amino acid, about–250 kDs) serine/threonine kinase known to control cell growth and regulate protein synthesis (Klann and Dever 2004; Hoeffer and Klann 2010). This hypothesis corroborates postmortem findings of significant reductions in mTOR, p70S6 K, eIF4B, and p-eIF4B protein expression in the PFC of MDD patients (Jernigan et al. 2011). Interestingly, polymorphisms in FK506 binding proteins that interact with the macrolide rapamycin and mTOR were found to be associated with increased recurrence of depressive episodes and with rapid response to ADT treatment, via effects on HPA axis regulation (Binder et al. 2004). Prior infusion of rapamycin into the medial PFC that disrupts the formation of mTOR complex 1 (mTOR

bound to the scaffolding protein, Raptor) abolished the synaptogenic and ADT activity of ketamine (Li et al. 2010, 2011a). Autry et al. (2011) suggest that the immediate effects of ketamine on the translational machinery within dendrites allows for the rapid synthesis of BDNF. Interestingly, this effect could possibly enhance synaptogenesis in the hippocampus, which would follow a time course (within hours) much faster than could be driven by classical ADTs (several weeks) (Nibuya et al. 1995; Coppell et al. 2003; Molteni et al. 2006; Martinowich et al. 2007; Russo-Neustadt et al. 1999). While this suggests that increased hippocampal BDNF and synapses are sufficient to produce ADT responses, the repercussions of such a fast process on neurogenic rates remain unknown.

The mTOR is a node of convergence of several signaling pathways that include the ones subserved by phosphoinositide-dependent kinase (PDK1), phosphatidylinositol 3 kinase (PI3 K), Akt (Protein kinase B), IGF1/2, and the tuberous sclerosis complex proteins 1 and 2 (Tsc1/2). The mTOR is thus influenced by allied upstream effectors, including mGluRs, NMDAR, AMPAR, BDNF/TrkB, and dopamine receptors (Hoeffer and Klann 2010). Which among them are specifically linked to ketamine's ADT properties is currently under investigation. As ketamine more closely influences NMDARs and AMPARs, they may well serve as a sine qua non for the drug's ADT activity. First, ketamine administration results in the insertion of the GluR1/2 subunits of the AMPAR into the membrane, which are coupled to synaptogenic proteins and whose activation likely contribute to neuroplastic and structural synapse modifications (Duman and Voleti 2012; Greer and Greenberg 2008). Indeed, the AMPAR antagonist NBQX prevented the rapid ADT-like behavioral and synaptogenic effects of ketamine (Li et al. 2010). Second, earlier studies report that NMDAR antagonists increase and decrease the discharge of pyramidal and GABAergic neurons, respectively (Homayoun and Moghaddam 2007), and rapidly enhance glutamate efflux in the PFC (Moghaddam et al. 1997; Lorrain et al. 2003; Zuo et al. 2006). In principle, the combined increase in AMPARs and blockade of NMDARs amidst increased glutamate tone, should allow for a spatiotemporal pattern of AMPAR (maximal) and NMDAR (minimal) activity that favor more efficient neurotransmission, as well as synaptogenesis. It is also tempting to speculate that such a pattern of NMDAR activity restricts optimal activation to synaptic NMDARs by increased synaptic glutamate, while constraining extrasynaptic NMDAR activity, a most conducive condition for synaptogenic processes (Hardingham and Bading 2010). Alternatively and in addition to a selective activation of discreet NMDAR subpopulations, Autry et al. (2011) suggest that ketamine could differentially affect NMDARs at different layers of neurotransmission. NMDARs can deliver both spontaneous at-rest signals [miniature excitatory postsynaptic currents (mEPSCs)] and action-potential (AP)-dependent signals, with opposite effects on protein synthesis, the former promoting it and the latter repressing it, as previously demonstrated (Sutton et al. 2004, 2007). They reasoned that it is precisely ketamine's blockade of mEPSCs that is sensed by eEF2, instructing it to switch off the inhibition on protein synthesis (Autry et al. 2011). In attempting to synthesize these data, one may hypothesize that ketamine's blockade on NMDAR-mediated mEPSC functions and/or extra-synaptic NMDARs

altogether would gate a broader effect of an AMPAR-mediated, intrinsic AP-dependent network activity. This broader effect should consolidate translational output, integrating and stabilizing it within the active neural network. It is yet to be determined if these mechanisms mediating ketamine's rapid ADT effects are similarly recruited by other potential fast-acters, including scopolamine (Furey and Drevets 2006), dextromethorphan (Lauterbach 2011), pindolol augmentation (Blier 2001) and DBS (Mayberg et al. 2005).

6.5 Region-Specific Modulation of Synaptogenesis by Stress and Antidepressants Points to Divergent Roles of Microcircuits in Depressive Behavior

It is noteworthy that stress in animal models has resulted in variable effects on synaptogenesis and microcircuit function among different areas (modules) of the limbic system. In the previous sections, we have mainly highlighted the adverse attenuating effects of stress on spine density and synaptic plasticity (LTP) in the PFC and the hippocampus. Counterintuitively, stress has been found to strengthen the synaptic connectivity and modify the spatiotemporal pattern of spine formation in amygdaloid spiny neurons (Mitra et al. 2005; Vyas et al. 2006; Segal et al. 2010). These observations corroborate human brain imaging studies that looked into modification in PFC-amygdaloid coupling in MDD patients (Heinz et al. 2005; Friedel et al. 2009). Chronic unpredictable stress or repeated social defeat has also increased spine density and dendritic arbors of NAc medium spiny neurons, along with an increase in neuronal excitability and mEPSCs (an index of increased glutamatergic synaptic input) (Rosenkranz et al. 2010; Vyas et al. 2003, 2004, 2006; Mitra et al. 2005), despite reduced NAc volume and accumbal function on reward processing (Pizzagalli et al. 2009; Wacker et al. 2009; Christoffel et al. 2011a, b). In the learned helplessness model, as well as in rats with congenital learned helplessness, ventral tegmental area (VTA)-projecting lateral habenular neurons also exhibit hyperexcitability driven by increased synaptic plasticity (Li et al. 2011a, b). These seemingly paradoxical growth and hyperactivity found in the amygdala, habenula, and the mesolimbic dopaminergic system are likely not a direct neurodegenerative consequence of glucocorticoids. They may rather represent an effect secondary to perturbations in the balance of activations and inactivations in the limbic neuronal network, precipitating abnormal activity-dependent expression of neurotrophic factors (BDNF). Indeed, while infusion of BDNF or neurotrophin-3 in the hippocampus yields ADT-like effects in the learned helplessness paradigm and in the forced swim test (Shirayama et al. 2002), when carried out in the VTA, depression-like behaviors were produced. Furthermore, after social defeat, BDNF is enhanced in the VTA-NAc pathway, paralleled by increased c-fos expression, stubby spine formation, and frequency of mEPSCs (increased synapse number) in these regions (Miczek et al.

2011; Christoffel et al. 2011a, b), although chronic fluoxetine administration increases BDNF mRNA and protein levels in the mesolimbic dopaminergic pathway (VTA and NAc shell), as well as in the PFC and hippocampus (Molteni et al. 2006). Recently, the inhibitor of kappa kinase (IKK), a downstream target of BDNF and cytokines, was also described having been significantly upregulated in the NAc following repeated social defeat in mice (Christoffel et al. 2011a, b). In the same vein, BDNF knockdown in these areas could produce ADT, rather than depressive behavior (Taliaz et al. 2009). For instance, while reduction of BDNF production and/or synaptogenesis within the hippocampus or the PFC have resulted in a depressive phenotype, inhibiting BDNF-TrkB signaling within the VTA and the NAc have led to ADT-like activity (Eisch et al. 2003; Berton et al. 2006; Krishnan and Nestler 2008, 2010; Nestler and Carlezon 2006). These findings may mean that the effect of stress and ADTs on neuronal architecture and activity depends upon the functional position of the brain areas within the neural network. As such, their presynaptic relationship with central hubs within this network (such as the PFC and the hippocampus), more primarily affected in MDD, will determine the structural/morphological, functional, and physiological impacts on these brain structures, and consequently, their behavioral output. For example, while stress generally decreases dendritic arborization and spine density in the medial PFC, a subset of infralimbic pyramidal neurons projecting to the basolateral amygdala is virtually unaffected (Shansky and Morrison 2009; Shansky et al. 2009), rendering it more susceptible to hyperdrive, hypertrophy, and hyperexcitability. Accordingly, ADT treatment, including DBS (Li et al. 2011b), could normalize these stress-induced abnormalities in these areas once signal propagation from central structures (the PFC and the hippocampus), is realigned. Furthermore, since chronic systemic or peripheral neurotrophic or growth factors elicit ADT effects (Aberg et al. 2000; Duman et al. 2009; Schmidt and Duman 2010), one might conclude that the effects on these central structures (the PFC and the hippocampus) predominate. However, since ADT intervention may not necessarily act directly on these areas as they do in the PFC and the hippocampus, some established effects may still prove resistant and irreversible even after symptom remission, a consequence that may be linked to spontaneous recovery and relapse of emotional disturbances in MDD. Altogether, these findings imply that region-specific modulation of synaptogenesis by stress and ADTs points to divergent roles (inhibitory or facilitatory) of different microcircuits on depressive behavior. It is not difficult to conceive that the circuitry modulating mood and related behaviors is primarily driven by central hubs, such as the PFC and the hippocampus, which directly or indirectly feed into all the other nodes within the network, each of which in turn controls different core behavioral features (anhedonia, helplessness/despair, etc.). The kind and severity of symptoms that may emerge as constituting the depressive phenotype may thus depend upon which microcircuits/nodes in the network are affected most profoundly.

7 The Interplay of Neurogenesis and Synaptogenesis in Antidepressant Action

7.1 Neurogenesis and Network Integration

Integration of previous stress episodes with novel ones invokes a precise orchestration of both neurogenic and synaptogenic processes in the PFC and/or the hippocampus. Indeed, even after neurogenic blockade, enhanced synaptogenesis and dendritic remodeling correlated with the therapeutic effect of ADTs, indicating that it is not neurogenesis alone, but also the activation and survival of already born cells into neuronal networks that are functionally important (Bessa et al. 2009). One question that warrants further investigation is with regard to the factors that allow for the effective integration/assimilation of new hippocampal neurons into the neural network. It is hypothesized that neurogenesis and neural integration increases the ability of the hippocampal network to accommodate and process increasing levels of complexity (Kempermann 2002). Such should be achieved without overloading the homeostasis of excitatory and inhibitory drives within the hippocampal, as well as the larger limbic network, as one could expect with promiscuous additions of neurons and synapses. An imbalanced information flow may then result, for example, in interference of selective retrieval/recall of old stored memories by the acquisition of new memories. To address this problem, a mechanism should be in place that would allow a fined-tuned spatial and temporal dynamics of neurogenic and synaptogenic processes for the systematic turnover and the proper integration of nascent neurons and synapses within the neural network. Here, much of the work done is in the domain of learning and memory, and several hypotheses have been suggested. This would include a constant adjustment of the gain of neurogenic rates (insertion of new ones) over apoptosis (eradication of old neurons). Once new neurons find their place, a number of possibilities could proceed: (1) a regulation of the intrinsic excitability (firing probability) of neurons within the network by changing the expression or functionality of ion channels involved in AP generation, (2) an equivalent modulation of the strength of synapses within the network by altering the expression of excitatory (glutamatergic) AMPARs (synaptic scaling), (3) neurogenesis of GABAergic interneurons matched to those of glutamatergic neurons to even out the net valence of neural networks, (4) neurogenesis of cells that cotransmit excitatory and inhibitory transmitters, (5) and the spontaneous alterations of the neurochemical identities of neurons, such as from an excitatory CA3 granule cells to inhibitory ones (for review, see Meltzer et al. 2005). Catastrophic consequences may arise when neuroplastic processes and network integration are significantly hampered, especially during the formative stages of the brain. This is much appreciated upon considering the sensitivity of critical developmental periods, during which neurogenesis and synaptogenesis are at hyperdynamic phases (Altman and Das 1965; He and Crews 2007). For instance, interfering with intrinsic systems known to participate in the regulation of juvenile neuroplasticity, such as

the pharmacological or genetic (knock-out) manipulations of the monoaminergic and endocannabinoid systems or by interfering with BDNF-TrkB signaling can precipitate long-lasting depressive/anxiety-like behaviors (Fiore et al. 2002; Karpova et al. 2009; Rubino et al. 2008; Bambico et al. 2010). These consequences are much less profound, if not at all insignificant when such conditions are introduced during adulthood, likely because neural networks are relatively stabler.

7.2 *Neurogenesis–Synaptogenesis Synergy: Toward the Path to Recovery*

We have discussed in this chapter that although there is limited cortical synaptogenesis and a slower turnover of new hippocampal neurons within the adult neural network, these are nevertheless recruited by ADTs, and hampered in depressive states. The relationship between synaptogenesis and neurogenesis in the ADT response is not very well described. We therefore propose a theoretical framework that would enable to reconcile the breadth of data on these neuroplastic processes. These processes are illustrated in Fig. 2. The proposed framework highlights that recovery can be achieved via different processes, organized in a sequential manner, as described below:

(1) ADT treatments can act on synaptogenesis in the PFC and/or the hippocampus, immediately eliciting therapeutic effects. Indeed, the PFC is necessary for the inhibition of negative cognitive bias, and both the PFC and hippocampus are integral in stress coping and adaptation, as well as in the regulation of the HPA axis. Therefore, restoring normal physiological PFC and hippocampal function may be sufficient to elicit symptomatic improvement.
(2) The increased function of glutamatergic efferents of the PFC could then modulate downstream monoaminergic neurotransmission. This process can also be achieved more directly via the use of compounds directly acting at monoaminergic synapses. In this case, however, the rapid recovery observed in step 1 is bypassed. In parallel, the functional recovery of PFC feedback over the HPA axis may lead to a decrease in glucocorticoid release from the adrenals, consequently moderating the neurotoxic effects that these hormones impose upon the hippocampus.
(3) Enhanced monoaminergic neurotransmission resulting from either the sequential progression of steps 1 and 2 or from the action of ADTs at monoaminergic transporters could induce, after several weeks of treatment, an increase in hippocampal neurogenesis. The decrease in glucocorticoid release that has also been induced after steps 1 and 2, should further contribute to the modulation of hippocampal neurogenesis.
(4) Once the nascent hippocampal neurons have integrated into the functional neuronal network, via increased synaptogenesis within the hippocampus, further improvements in HPA regulation can be expected. This would also

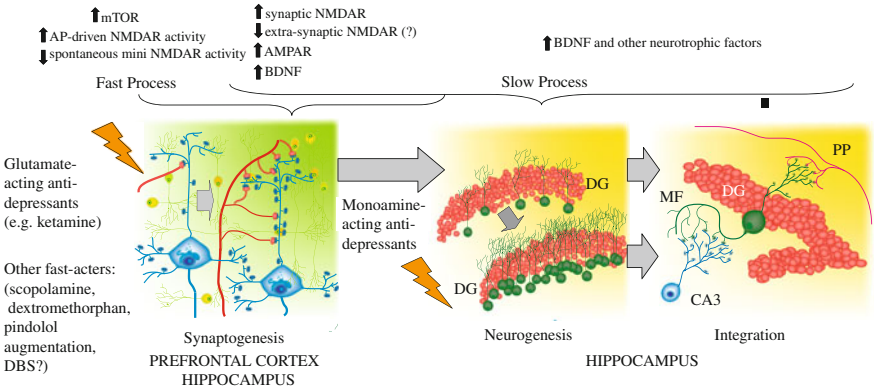

Fig. 2 The ability of antidepressants to normalize network information flow is underpinned by neurobiological events that progress within specific time windows. These events include an initial phase that corresponds to a fast synaptogenic enhancement (increased dendritic arborizations and spine density) in the PFC and hippocampus; and a slower maintenance/stabilization phase that corresponds to an increase in hippocampal neurogenesis, followed by the integration of nascent neurons within the hippocampal network and the larger limbic network. The fast synaptogenic process could be instigated by putative rapid-acting antidepressants, such as ketamine (possibly scopolamine, pindolol augmentation, dextromethorphan, and DBS). These treatments could act on the mTOR pathway, on BDNF, NMDA receptor and/or AMPA receptor activity. Conventional antidepressants, such as monoaminergic agents, elicit their antidepressant effects by directly enhancing hippocampal neurogenesis (in the DG), and possibly hippocampal synaptogenesis (in the CA1 and CA3 subfields), but at a much slower rate, via a slow-progressing enhancement of neurotrophic factors (BDNF). *AMPAR*, 2-amino-3-(5-methyl-3-oxo-1,2- oxazol-4-yl)propanoic acid) receptor; *AP*, action potential; *BDNF*, brain-derived neurotrophic factor; CA3, cornu ammonis region 3; *DBS*, deep brain stimulation; *DG*, dentate gyrus; *MF*, mossy fiber; *mTOR*, mammalian target of rapamycin; *NMDAR*, N-methyl-D-aspartic acid receptor; *PP*, perforant pathway

facilitate recovery via restoration of normal function in different projection areas through the connectivities of the hippocampus with the PFC, the hypothalamus, the NAc and the amygdala, and the rest of the limbic network.

This model proposes that PFC synaptogenesis, and hippocampal neurogenesis and synaptogenesis may all contribute to recovery from MDD. Each of these processes has its specific time window. Synaptogenesis appears to be more sensitive to ADTs, and is therefore harnessed much earlier than neurogenesis, which is a naturally slower process (Hajszan et al. 2005; Li et al. 2010; Autry et al. 2011) (Fig. 2). Synaptogenesis in the PFC, as well as in the hippocampus, may be rapidly instigated (within hours) in a BDNF- and mTOR-dependent manner by glutamate-modulating putative ADTs (such as ketamine) (Autry et al. 2011; Li et al. 2010, 2011a) and possibly by other fast-acters. This could also be instigated by conventional ADTs (such as fluoxetine) though at a much slower rate. The SSRI fluoxetine induces increased synaptogenesis in the rat hippocampal CA1 and CA3

subfields after 5 days of daily administration (Hajszan et al. 2005), and chronic but not acute electroconvulsive seizures increases expression of mTORC1 components in both the PFC and the hippocampus (Elfving and Wegener 2012). These effects on mTORC1 and synaptogenesis may be mediated by a much slower but stabler increase in BDNF (Liu et al. 2011; Slipczuk et al. 2009), which in turn could evidently be seen after chronic but not after acute administration of conventional ADTs (Sairanen et al. 2007). The potency of ADT response through the fast process (Fig. 2) could last up to a week in treated animals (Autry et al. 2011; Duman and Voleti 2012) and MDD patients (Zarate et al. 2006; Zarate et al. 2010). It is not certain whether repeated ketamine treatment could induce a neurogenesis-dependent ADT activity, but this is of less value to the clinical setting, as this "club drug" is known to be addictive and psychotomimetic. Nevertheless, longer lasting mood and cognitive recovery are suggested to develop in a neurogenesis-dependent manner (Pinheiro et al. 2011). Therefore, it may be conceived that synaptogenic activity could represent an initial phase of ADT action. In so far as the resultant increase in synaptic strength can be maintained within a given time window, it can potentially gate broader and sustained effects on neurotransmission, that in turn could trigger more stable increases in neurotrophic (BDNF) production/signaling in an activity-dependent manner. Consequently, this would induce an increase in hippocampal neurogenic rates, inter-network integration of new neurons, reorganization of the larger limbic network, and a more stable recovery and maintenance. This model remains speculative, but could provide the impetus for further experimental investigations.

8 Summary and Conclusion

The complexity of the pathophysiology of MDD could be understood as a hierarchy of dysfunctions: cognitive and emotional information bias; imbalance in the neuroendocrine response; disarray in intracellular and molecular signaling essential to the production of neurotrophic factors; abnormalities in neuronal/cytological elements and neuroplasticity, including impairments in spinogenesis and synaptogenesis, and in neurogenesis; and genetic and epigenetic anomalies. These dysfunctions, however, tend to reinforce each other as they manifest. The preponderance of clinical and animal data has encouraged us to infer about the molecular underpinnings of the cognitive, behavioral, and emotional symptoms seen in MDD. These efforts have paved the way to the development of several insightful theories. From among these, the recent revitalization of a network hypothesis has emerged as an attempt at bridging and integrating basic, neurobiological theories of MDD with the clinical and phenomenological characterization of the disease. In this chapter, we have sought to examine how impairments in cortical synaptogenesis and hippocampal neurogenesis, which are precipitated by a number of external and internal factors, could lead to perturbations in the flow of information within the limbic neural network. Normal neurotransmission

coursing through different hubs within this network maintains a balance of activation and inactivation of limbic structures controlling unique aspects of the stress response. Under healthy conditions, this balance adjusts accordingly depending upon the stress demand and on allostatic load. Under depressogenic conditions, such as chronic exposure to stress, it may be rendered off-kilter as the accumulative deteriorative influences on synaptogenesis and neurogenesis impose proportional effects on signal transmission through the neural network. ADTs overcome these adversities encountered by the limbic system, by reversing the damage to neurons. There is a growing body of data suggesting divergent molecular mechanisms mediating this action of ADTs. Increased attention has been directed on those that are responsible for the fast-acting therapeutic effects of experimental treatments, such as glutamate-acting agents, in light of the fact that virtually all clinically used ADT drugs exert slow onset of therapeutic action. One such mechanism involves the activation of intracellular signaling cascades that control translational processes leading to cortical, but also hippocampal, synaptogenesis. ADT-induced synaptogenesis may thus represent an initial process that sets the stage to a broader and more persistent expression and maintenance of the ADT response. Indeed, the increase in the number of dendritic spines and synaptic contacts in the PFC and hippocampus could enhance excitatory transmission propagating down subcortical limbic areas, which may subsequently modulate monoaminergic transmission in different areas, including the hippocampus. This sequence of events may serve instrumental in strengthening other neurobiological trajectories that enhance the ADT response, and accordingly maintain its expression for longer periods. In this respect, it is in this second phase when hippocampal neurogenesis ensues, that it becomes pivotal. Indeed, the action of chronic monoaminergic ADT treatment is blocked when nascent hippocampal neurons have been suppressed, an effect that does not progress if only neuroblasts have been suppressed. Moreover, the therapeutic effects of these drugs on hippocampal neurogenesis requires that newborn hippocampal neurons are inserted within a functional neuronal network, a process lasting several weeks. On the other hand, the generation of new synapses in the PFC could be achieved within hours, suggesting that an effect on synaptogenesis alone is not sufficient to obtain a stable and long-lasting recovery. On the contrary, the duration and severity of MDD may be associated with the extent by which hippocampal processes, which may likely include neurogenesis, are adversely compromised.

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Deficient Plasticity in the Hippocampus and the Spiral of Addiction: Focus on Adult Neurogenesis

Juan J. Canales

Abstract Addiction is a complex neuropsychiatric disorder which causes disruption at multiple levels, including cognitive, emotional, and behavioral domains. Traditional biological theories of addiction have focused on the mesolimbic dopamine pathway and the nucleus accumbens as anatomical substrates mediating addictive-like behaviors. More recently, we have begun to recognize the engagement and dynamic influence of a much broader circuitry which encompasses the frontal cortex, the amygdala, and the hippocampus. In particular, neurogenesis in the adult hippocampus has become a major focus of attention due to its ability to influence memory, motivation, and affect, all of which are disrupted in addiction. First, I summarize toxicological data that reveal strongly suppressive effects of drug exposure on adult hippocampal neurogenesis. Then, I discuss the impact of deficient neurogenesis on learning and memory function, stress responsiveness and affective behavior, as they relate to addiction. Finally, I examine recent behavioral observations that implicate neurogenesis in the adult hippocampus in the emergence and maintenance of addictive behavior. The evidence reviewed here suggests that deficient neurogenesis is associated with several components of the downward spiraling loop that characterizes addiction, including elevated sensitivity to drug-induced reward and reinforcement, enhanced neurohormonal responsiveness, emergence of a negative affective state, memory impairment, and inflexible behavior.

Keywords Plasticity · Addiction · Adult hippocampal neurogenesis · Drugs of abuse · Behavior

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Curr Topics Behav Neurosci (2013) 15: 293–312
DOI: 10.1007/7854_2012_230

Published Online: 11 September 2012

Contents

1 Introduction

Few human diseases can disrupt human behavior as profoundly as drug addiction. Emotion, cognition, and behavioral processes all become dysregulated in a spiral of recurrent thoughts and compulsive actions directed at procuring and taking drugs (Hyman and Malenka 2001; Koob and Kreek 2007; Gardner 2011; Weiss et al. 2001). Such general alteration of psychological processes is caused by the widespread actions of drugs at numerous levels within the neuroendocrine system and the persistent changes evoked at molecular, biochemical, and systems levels (Nestler 2005; Robison and Nestler 2011; Kalivas 2008; Kalivas and Volkow 2005). Traditional psychobiological theories of addiction have focused mainly on the basal ganglia and the prefrontal cortical network as substrates that undergo neuroadaptive change during drug exposure (Robinson and Berridge 1993; Wise 1987). Specifically, the research accumulated in the last 30 years has pointed at the nucleus accumbens and the neurotransmitter dopamine as the key players that mediate not only the stimulant, rewarding and reinforcing effects of addictive drugs, including alcohol, opiates, and psychomotor stimulants, but also some of the long-term effects of drug exposure, including withdrawal (Koob and Weiss 1992; Pulvirenti and Diana 2001; Volkow et al. 2011). In parallel, however, increased attention has been devoted to the pathways that connect the limbic lobes with the basal ganglia, linking systems subserving emotion and memory, such as the amygdala and the hippocampus, with the abnormal action patterns that typify addiction. As a result, we are beginning to recognize that the deficits in executive, motivational, emotional, and mnemonic function that characterize addiction, which derive from maladaptive neuroplasticity in multiple brain regions and neurotransmitter systems, are more widespread than previously believed (Canales 2007, 2010; Eisch and Harburg 2006).

Addictive drugs have the ability to alter adult neurogenesis, one of the most remarkable forms of physiological plasticity and functional modifiability in the brain. In the dentate gyrus of the adult hippocampus of many animal species, including man, a multistep process leads to the generation of new granular neurons throughout life (Abrous et al. 2005; Alvarez-Buylla and Lim 2004). Addictive

drugs provoke alterations in the neurogenic process, inducing changes in the proliferative capacity of progenitor cells and/or the development and fate of neuronal precursors. Initial evidence was obtained for morphine, heroin, and methamphetamine, with studies investigating the effects of other drugs of abuse, including alcohol, cocaine, and ecstasy (MDMA), soon following (Eisch and Mandyam 2004; Canales 2007; Eisch et al. 2008). This initial evidence suggested that in addition to the basal ganglia-neocortical loop pathways, the hippocampus may be part of a wider circuitry dynamically engaged to modulate addictive behavior. Drug-induced impairments in adult hippocampal neurogenesis may influence several psychological processes that are affected by drug exposure. Such conjecture rests on evidence suggesting that adult-born neurons may play a role in learning, memory, and mood regulation, on the one hand, and influence specific drug-related behaviors, on the other (Canales 2007, 2010; Abrous et al. 2005). Here, I will summarize toxicological research on the effects of addictive drugs on neurogenesis, critically examine the possible implications of drug-induced impairments in hippocampal neurogenesis across the domains of cognition, emotion, and behavior, and evaluate how these deficits may contribute to generate and perpetuate drug-seeking behavior.

2 Drug Toxicology

Ample evidence indicates that exposure to addictive drugs regulates the rate of neurogenesis in the hippocampus, suggesting a possible role of neurogenesis in the induction of the long-term behavioral, emotional, and cognitive changes that drugs elicit. I will briefly summarize the most relevant studies that have investigated drug effects on neurogenesis. More extensive reviews on this subject are already available (Eisch et al. 2008; Canales 2007, 2010; Eisch and Harburg 2006). One of the first drugs to be characterized in this regard is alcohol. Using a semi-chronic (4-day) binge paradigm, initial data revealed that gavaged alcohol reduced proliferation and survival of newborn cells in the dentate gyrus (Nixon and Crews 2002). Additional experiments showed a protracted compensatory burst of neurogenic activity after binge alcohol exposure and withdrawal, with enhanced proliferation being observed 1 week after discontinuation of alcohol treatment (Nixon and Crews 2004). Later observations in rats that were fed a liquid diet containing moderate alcohol levels during 6 weeks showed much reduced survival of neuronal progenitors, increased apoptosis in the dentate gyrus, and attenuation of these neurotoxic effects by the antioxidant agent, ebselen (Herrera et al. 2003). Further, chronic self-administration (2- and 4-week exposure) of alcohol in rats inhibited proliferating nuclear antigen (PCNA) expression, decreased survival of 2,5-bromodeoxyuridine (BrdU) labeling, and significantly reduced the size of the dendritic arbors of double cortin-labeled immature differentiating neurons (He et al. 2005), suggesting that alcohol produces severe disruption of neurogenic processes in the hippocampus. In addition, semi-chronic (8-day) alcohol self-administration in mice reduced neural stem cell

proliferation in the dentate gyrus, whereas wheel running produced opposite effects, with the combination of both alcohol self-administration and exercise having enhancing effects on neurogenesis (Crews et al. 2004). Moreover, the deleterious effects of chronic alcohol exposure have been replicated in nonhuman primates. Long-term binge (11-month) alcohol intake persistently reduced hippocampal proliferation and neurogenesis, affecting both proliferative capacity and migration of hippocampal preneuronal progenitors (Taffe et al. 2010).

Negative regulation of hippocampal neurogenesis by opiate exposure was shown following chronic administration of morphine, and after heroin self-administration, in the rat (Eisch et al. 2000). Treatment with opiates such as morphine has deleterious effects on hippocampal neurogenesis. Morphine exposure during 24, 72, and 96 h decreased the number of S-phase and total cycling cells and impaired the progression of progrenitor cells to mature stages of development (Arguello et al. 2008, 2009). Data suggested that high levels of circulating morphine leading to behavioral dependence, rather than pulsatile exposure to the drug, are required to downregulate proliferation in the dentate gyrus (Fischer et al. 2008). Repeated morphine treatment and withdrawal produced a reduction in cell proliferation and polysialylated forms of neural cell adhesion molecule (PSA-NCAM) expression in the dentate gyrus, and alteration of neuronal phenotypes, with enhanced transcription of glutamate decarboxylase-67 mRNA in granular neurons (Kahn et al. 2005).

Psychomotor stimulants, including cocaine and amphetamine-like substances, also reduce neurogenesis in the hippocampus. Detailed studies on the effects of cocaine treatment on proliferation, maturation, and survival of newborn hippocampal cell revealed that semi-chronic (8-day) and chronic cocaine (24-day) exposure decreased proliferation rates without altering the survival and growth of immature cells, or disrupting the synaptic architecture of the mossy fiber projection system that connects the dentate gyrus with the CA3 region (Dominguez-Escriba et al. 2006). These findings extended the initial observation of a decrease in proliferation of granule cells following chronic (14-day) cocaine exposure (Yamaguchi et al. 2004). Recent data suggest that the deleterious effects of repeated cocaine treatment on hippocampal cellular proliferation may be prevented by upregulation of protein kinase C-mediated signal transduction (Xie et al. 2009). Also, high dose methamphetamine treatment in gerbils produced a decrease in granule cells proliferation, with BrdU-labeled cells gradually declining from the septal toward the temporal pole of the hippocampus (Teuchert-Noodt et al. 2000). This effect resulted from single acute exposure to methamphetamine. The negative effects of methamphetamine may be mediated, at least in part, through oxidative and nitrosative stress, as demonstrated in neural progenitor culture systems (Venkatesan et al. 2011). However, long-term (14-day) *d*-amphetamine treatment produced no changes in progenitor cell proliferation but decreased the survival of neuronal precursors after 4 weeks of withdrawal (Barr et al. 2010). Another stimulant, caffeine, was found to reduce proliferation in the dentate gyrus in rats, but only if administered in the light phase of the sleep cycle (Kochman et al. 2009), with similar results being obtained for the wake-promoting agent,

modafinil, suggesting that the effects of stimulants of granule cell proliferation may be partly due to their sleep suppressive effects. The effects of caffeine appear to affect only the proliferative capacity of neuronal progenitors, with moderate doses administered semi-chronically (7-day) producing decreases in cell proliferation but not in survival and differentiation of newly generated hippocampal neurons (Wentz and Magavi 2009).

Substances, such as nicotine, marijuana, and hallucinogenic drugs, including 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy"), have also been investigated. Chronic nicotine self-administration decreased the expression of PSA-NCAM, enhanced cell death, and diminished neurogenesis in the dentate gyrus (Abrous et al. 2002). The effects of different administration regimens of Δ^9-tetrahydrocannabinol (THC), the main active component of marijuana, have been studied. THC did not produce changes in proliferation in the dentate gyrus following acute, sequential, or chronic escalating exposure at doses that induced severe intoxication (Kochman et al. 2006). However, in female rats, chronic (10-day) escalating exposure to THC during adolescence did produce reductions in the number of BrdU-positive cells in the dentate gyrus as well as in hippocampal volume (Realini et al. 2011). On the other hand, binge treatment (8 treatments at 6 h intervals) with the psychedelic drug, MDMA, did not alter cell division in the dentate gyrus but compromised the survival of newborn cells labeled before the treatments, suggesting that MDMA is toxic to vulnerable hippocampal precursors (Hernandez-Rabaza et al. 2006). Recent observations further indicated that binge-like treatment (ten treatments at 6 h intervals) with alcohol, MDMA, and combinations thereof all reduced survival of neural precursors in the dentate gyrus, without affecting the number and length of the dendritic arbors of maturing neurons (Hernandez-Rabaza et al. 2010).

A critical question is the extent to which drug effects on neurogenesis are persistent over time, or even permanent, and whether these effects, if present, have any behavioral relevance. Evidence in this regard is still very scarce. Exposure to drugs during embryogenesis can indeed have lasting effects on brain function, including neurogenesis. Prenatal alcohol treatment blocked in mice the normal increase in neurogenesis observed in controls following exposure to an enriched environment (Choi et al. 2005). Early postnatal alcohol exposure also produced persistent deficits in neurogenesis observed at PD50 and PD80 (Klintsova et al. 2007). Combined prenatal and perinatal exposure to MDMA similarly produced long-lasting effects on neurogenesis (i.e., reduced proliferation rates), which were apparent well into adulthood (Cho et al. 2008). However, the long-term effects of drug exposure during adulthood might be different. Regeneration of brain, including generation of many new neurons in the dentate gyrus, occurs in adulthood during abstinence after binge ethanol treatment (Nixon and Crews 2004; Nixon et al. 2008), and this is likely to occur following chronic exposure, and abstinence from, other drugs of abuse. Thus, unless severely affected by drug exposure during development, the brain retains the ability to resume the normal mechanisms of proliferation, differentiation, and integration of adult-generated hippocampal neurons. It has been suggested that such ability may be important for

promoting recovery and providing resistance to relapse (Mandyam and Koob 2012; Nixon and Crews 2004).

The data reviewed in this section provides compelling evidence demonstrating that addictive drugs negatively regulate plasticity in the adult hippocampus. Such findings suggest an association of neurogenesis with at least some correlates of addiction, which span through different functional psychological and behavioral domains, including learning and memory function, regulation of affective states and vulnerability.

3 Neurogenesis in the Hippocampus and the Learning Domain

The incorporation of adult-born neurons to the granular layer of the DG throughout the lifespan is one of the most remarkable forms of plasticity that occurs in the brain. Such modifiability is consistent with the fundamental nature of memory formation, which involves the acquisition of novel information linked to persistent physiological changes in neural activity. Newborn hippocampal cells exhibit special electrophysiological properties that enhance their ability to undergo change, including enhanced synaptic excitability and lower threshold for long-term potentiation (LTP) induction at early maturational stages (Snyder et al. 2001), which supports a role of adult neurogenesis in memory formation. In line with these observations, the induction of LTP at perforant path-granule cell synapses can stimulate proliferation and facilitate the long-term survival of adult-born hippocampal neurons (Bruel-Jungerman et al. 2006). Associative LTP is more easily induced in young neurons than in mature neurons, with T-type Ca^{2+} channels generating Ca^{2+} spikes and boosting fast Na^{+} currents that facilitate synaptic plasticity (Schmidt-Hieber et al. 2004).

There are still some controversies surrounding the involvement of hippocampal neurogenesis in learning and memory. In some learning paradigms, experimental studies have yielded data in favor and against a contribution of hippocampal neurogenesis to memory function (Leuner et al. 2006). Moreover, adult hippocampal neurogenesis shows much variability across species and declines steadily with age (Amrein and Lipp 2009), which argues against an essential role of neurogenesis in cognition. However, consensus is beginning to emerge on a number of cognitive functions that require active engagement of hippocampal neurons. Some of these functions may influence, directly or indirectly, addictive behaviors. Drug abuse impacts negatively on at least four broad areas of cognitive function that may be modulated by adult hippocampal neurogenesis: contextual memory, spatial memory, working memory, and cognitive flexibility (Canales 2010). The deficits described in drug addicts in the areas of episodic memory, contextual learning, spatial memory, working memory, and mental/cognitive flexibility could result, at least in part, from impaired adult neurogenesis and long-term neurotoxicity at hippocampal synapses.

The hippocampus is involved in forming contextual representations of the space in which experiences take place, including aversive experiences and hedonic effects induced by drugs. Selective lesions of the dentate gyrus induced by the neurotoxin, colchicine, prevented in rats context-conditioned fear and cocaine-induced place preference (Hernandez-Rabaza et al. 2008). It is plausible that the deficits in episodic memory exhibited by stimulant addicts may ultimately result from failure to acquire or retrieve contextual information. Could this failure be related to the disruption produced by drug abuse on adult hippocampal neurogenesis? Convincing animal work demonstrates that loss of neurogenesis impairs contextual learning. For example, exposure to brain irradiation and subsequent ablation of adult hippocampal neurogenesis blocked in rats the establishment of associations between contextual cues and shock, while sparing cue-conditioned fear, which does not require the integrity of the hippocampus (Winocur et al. 2006; Wojtowicz et al. 2008; Hernandez-Rabaza et al. 2009). Research in rats has further revealed that reduced neurogenesis impairs spatial performance when stimuli to be remembered are presented with little spatial separation, but not when they are apart (Clelland et al. 2009). Thus, the ability of drugs to disrupt neurogenesis could lead to detrimental effects on episodic memory by altering the ability of the hippocampal network to separate events, put them into context, and chunk them into meaningful episodes of information.

Second, it is likely that impairments of hippocampal neurogenesis following chronic drug exposure have a negative impact on spatial abilities and working memory performance in humans. In neuropsychological assessments, drug addicts suffer from deficits in visuospatial memory and working memory performance, which may be partially associated with neocortical para hippocampal and hippocampal dysfunction (Canales 2010). However, it seems that simple forms of spatial information processing are not affected by deficits in adult neurogenesis. The acquisition of a water maze task was not altered in mice treated with the alkylating agent temozolomide, which reduced hippocampal neurogenesis (Garthe et al. 2009). Similarly, focal hippocampal irradiation or genetic ablation of hippocampal neurogenesis in mice spared spatial learning in the Morris maze and the Y-maze tests (Saxe et al. 2006), and irradiation in rats had no effect on spatial learning (Wojtowicz et al. 2008). One possible role of newborn hippocampal neurons may be the long-term consolidation and/or retrieval of spatial information, which can be evaluated time after task acquisition. Experiments performed in both mice and rats support that adult hippocampal neurogenesis contributes to the long-term retention of spatial information. Transgenic mice with transiently reduced population of adult-born neurons showed impairments in the long-term retention of a spatial task (Deng et al. 2009), as did irradiated rats (Snyder et al. 2005). Further, exposure to a cytostatic agent which caused depletion of young hippocampal neurons impaired in mice remote, but also recent, memory in two spatial tasks (i.e., Morris water maze and object location tasks), while sparing memory whose integrity does not depend on the hippocampus (Goodman et al. 2010). Moreover, spatial learning appears to induce reorganization of hippocampal networks, with circuits being sculpted by both regulated selection and elimination of different

populations of neurons (e.g., survival of relatively mature neurons, apoptosis of more immature cells, and enhanced proliferation of neuronal precursors) (Dupret et al. 2007). Spatial learning also enhances the maturation of dendritic processes of adult-generated hippocampal neurons (Tronel et al. 2010). Thus impairments of hippocampal neurogenesis induced by chronic drug exposure could have a negative impact on spatial abilities in humans. In addition, an important function of the dentate gyrus is to process working memory in spatial tasks (Hernandez-Rabaza et al. 2007). The study of working memory function in animals with suppressed neurogenesis has yielded controversial results. Irradiation in rodents was reported to impair working memory in the Morris water maze task (Winocur et al. 2006), whereas others have found no effect of irradiation on a delayed matching to place task in the T-maze (Hernandez-Rabaza et al. 2009). Other laboratories have reported that hippocampal irradiation produced an enhancement of working memory performance when the same information was shown repeatedly in the radial maze (Saxe et al. 2007). Such discrepancies could stem from confounding effects of stress/anxiety associated with the memory tasks (e.g., Morris water maze task), which may interfere with performance of complex tasks (e.g., difficult working memory tasks), while not affecting or even facilitating simpler tasks (Diamond et al. 2007). Given that drug addiction is associated with elevated levels of stress and anxiety, drug effects on hippocampal neurogenesis may predispose individuals to working memory impairment.

Third, chronic drug abuse can lead to cognitive bias, including mental rigidity (i.e., lack of flexibility, cognitive stereotypy). In animals such deficits could manifest themselves as an inability to reverse previously learned behavioral patterns, a difficulty in extinguishing behaviors or a preference for the use of stimulus-response strategies, at the expense of hippocampal (i.e., cognitive, spatial) strategies. The role of neurogenesis in modulating the ability to adaptively reverse learning strategies is presently unclear. After pharmacological reduction of adult neurogenesis, mice showed deficits in spatial reversal in the water maze task (Garthe et al. 2009), but irradiated rats showed no impairment in shifting previously learned rules in a T-maze (Hernandez-Rabaza et al. 2009). Extinction behavior, however, appears to be affected by reduced hippocampal neurogenesis. Mice with impaired neurogenesis displayed deficits in the extinction phase of a spatial preference and a contextual fear response (Deng et al. 2009), as did rats following drug self-administration (Noonan et al. 2010). Stress can strongly influence learning. Chronic social stress has been shown to decrease double cortin expression in the hippocampus and to promote the use of habit-based learning strategies, which depend on basal ganglia activation, instead of hippocampal (i.e., spatial) strategies (Ferragud et al. 2010). As indicated above, one of the suggested roles of adult hippocampal neurogenesis is to facilitate spatial separation (Clelland et al. 2009). Inducible genetic expansion of the population of adult-born neurons through enhancing their survival produced in mice selective effects on learning. Mice exhibited normal object recognition, spatial learning, contextual fear conditioning, and extinction learning, but enhance ability to discern between overlapping contextual representations, which requires efficient pattern separation

(Sahay et al. 2011). Deficient pattern separation may induce cognitive rigidity. Mice with reduced neurogenesis displayed difficulties in switching from a previously established spatial avoidance response to a novel response that deviated slightly from the former (Burghardt et al. 2012). Taken together, these data suggest that a weakened hippocampal neurogenesis, and a subsequently reduced influence of the hippocampus on the basal ganglia-neocortical systems, may generate inflexible behaviors. In the context of addiction, such weakened neurogenesis could contribute to perpetuate drug-seeking behavior when alternative, more adaptive options may be available.

In summary, the experimental work in the area of learning and memory function reveals that the negative effects of chronic drug exposure on hippocampal plasticity may disrupt contextual/episodic memory formation, alter spatial abilities, induce deficits in working memory function and promote mental rigidity, a cognitive feature that may increase vulnerability to drug addiction.

4 Neurogenesis in the Hippocampus and Regulation of Affective States

There is a significant body of evidence indicating that the hippocampal network is one of the neural substrates that regulates motivation, mood, and affective behavior. Drug addiction is a psychiatric disorder typically associated with dramatic changes in such functions. Specifically, neurogenesis in the adult hippocampus has gained considerable attention as a substrate for the regulation of mood and the emergence of pathological affective disorders (Lucassen et al. 2010; Dranovsky and Hen 2006; David et al. 2010; Warner-Schmidt and Duman 2006; Petrik et al. 2012). Due to its sensitivity and vulnerability, the hippocampus has been a focus of research into the pathophysiology of stress and stress-related syndromes. Chronic stress is known to induce atrophy of the projection field of the mossy fiber system that connects the dentate gyrus with the CA3 subfield (McEwen and Magarinos 2001). In this context, there is evidence that chronic exposure to unpredictable stress produces impairments in hippocampal neurogenesis, increases the expression of anxiety- and depressive-like behaviors, and induces significant learning and memory deficits (McEwen and Magarinos 2001; McEwen 2010), while chronic predictable stress may have opposite effects (Parihar et al. 2011). Such findings support the view that neuroplasticity phenomena in the hippocampal region are involved in the pathophysiology of mood disorders and may possibly lead to dysregulation of emotional processes in addiction.

Evidence has shown that chronic, but not acute, antidepressant treatment increases in rats the number of newborn cells in the dentate gyrus and hilus regions of the hippocampus, with a time course that resembles the pattern of therapeutic action in humans (Malberg et al. 2000). Further research has shown that the

efficacy of the antidepressant, fluoxetine, is limited to some behavioral paradigms and tasks in irradiated mice treated with corticosterone (David et al. 2009). Further, animals exposed to inescapable shock in the learned helplessness model of depression showed a decreased proliferation in the dentate gyrus, which was blocked by treatment with fluoxetine (Malberg and Duman 2003). In nonhuman primates, separation stress resulted in depression-like behaviors (e.g., anhedonia and subordinance) and impaired neurogenesis. Both deficits were prevented by fluoxetine treatment, which required ongoing neurogenesis to exert therapeutic effects (Perera et al. 2011), confirming previous observations (Santarelli et al. 2003). The finding that antidepressant medication enhances neurogenesis has recently been extended to human hippocampal progenitor cells, adding validity to the findings obtained using animal models. Exposure to the antidepressant, sertraline, enhanced human neurogenesis via glucorticoid-dependent mechanisms (Anacker et al. 2011). Altogether considered, these observations suggest that enhanced neurogenic activity induced by chronic antidepressant treatment may contribute to reverse, or at least partially attenuate, the maladaptive changes that occur in the hippocampus as a result of chronic stress.

Neurogenesis occurs both in the dorsal and ventral dentate giri of the hippocampus, corresponding to the septal and temporal regions, respectively. The densities of primary and amplifying progenitor and young newborn neurons are higher in the dorsal than in the ventral dentate gyrus, and neurogenesis appears to decline at a faster rate in the ventral than in the dorsal hippocampus (Jinno 2011). Dorsal and ventral regions of hippocampus are believed to mediate segregated functions, with the dorsal aspect being primarily involved in spatial information processing and the ventral in the regulation of motivation and emotion (Bannerman et al. 2004; Fanselow and Dong 2010; Kheirbek and Hen 2011). Recent evidence suggests that treatment with lithium, a mood stabilizer, enhanced proliferation rates selectively in the ventral dentate gyrus in mice exposed to immobilization stress (O'Leary et al. 2012). In this context, it is important that future work examines neurogenesis in the ventral hippocampus as a substrate for drug-induced dysregulation of affective behaviors.

Drugs of abuse severely affect mood regulation and hypothalamo-pituitary adrenal (HPA) axis responsivity (Lovallo 2006; al'Absi 2006). For example, greater stress-induced, but not drug-induced, craving was observed in patients who exhibited shorter time to relapse, with corticotropin and cortisol responses predicting higher cocaine intake in a 90-day follow-up (Sinha et al. 2006). Similar associations with intensity of drug-induced reward and relapse have been observed for other major drugs of abuse, including nicotine and alcohol (McKee et al. 2011; Sinha et al. 2011). Altered functioning of the HPA axis resulting from chronic drug exposure and psychosocial stresses associated with drug abuse may provide insights into the nature of motivational processes that go awry in addiction. In addition, stress-induced dysregulation of the corticotrophin and HPA system appears to play a role in the development of addiction and vulnerability to relapse (Sinha 2008; Koob 2010). These effects could be mediated, at least partially, by drug-induced inhibition of hippocampal neurogenesis. It is known that prenatal

stress in rats produces lifelong reduction of neurogenesis in the dentate gyrus and lasting alterations in hippocampal-related spatial tasks (Lemaire et al. 2000). It appears that the level of circulating glucocorticoids plays a critical role in shaping neurogenic processes in the hippocampus. Permanent suppression of glucocorticoid secretion from midlife to senescence led to increased neurogenesis in aged rats and attenuated cognitive decline, while chronic upregulation of HPA axis decreased hippocampal cell proliferation and induced memory impairments in aged rats (Montaron et al. 2006). Recent data also suggested that suppression of adult neurogenesis potentiated neurohormonal responsiveness to a mild stressor (Schloesser et al. 2009). Moreover, consistent with a role of the hippocampus in the regulation of HPA axis responsiveness, results showed that glucocorticoid levels were slower to recover after moderate stress in neurogenesis-deficient mice, which also showed a stress-like behavioral phenotype, including enhanced food aversion after acute stress and anhedonia (Snyder et al. 2011). These observations and those of Surget and cols., who reported that antidepressant treatment reverses the stress-induced impairment of HPA axis, have led to the hypothesis that newly generated neurons in the hippocampus may act as a buffer system for stress coping, allowing the hippocampus to take control of central stress systems and recover general function (Snyder et al. 2011; Surget et al. 2011).

5 Neurogenesis and Addiction-Related Behaviors

While research on the impact of addictive drugs on the physiology and dynamics of hippocampal neurogenesis has grown over the last decade, comparatively fewer studies have addressed in animal models the role of adult-generated neurons in mediating specific behavioral processes associated with addiction. Thus, compelling evidence demonstrates that repeated exposure to drugs, such as alcohol, opiates, and psychomotor stimulants, severely impairs one or several of the processes associated with adult hippocampal neurogenesis. However, there are considerable gaps in our understanding of the behavioral consequences linked to this impairment. Yet, some critical findings have already been reported clearly suggesting that neurogenesis in the adult hippocampus plays a part in the spiral of addiction (Fig. 1). Addiction is a multifaceted syndrome and many aspects contribute to its development and persistence. Drugs of abuse share with natural reinforcers, such as food and sexual contact, the ability to produce reward, strengthen stimulus-response associations, elicit repetitive patterns of behavior and form drug-taking habits (Canales 2005; Everitt et al. 2008; Robbins and Everitt 2002). Withdrawal from drugs can also generate negative affect and dysphoria, which can also contribute to generate drug-seeking responses to alleviate those symptoms. After extended withdrawal from drugs, preoccupation and anticipation of drug effects also provokes craving, a major force driving relapse to drug-seeking (Koob 2000, 2010; Weiss 2010). Recent theories of addiction are beginning to incorporate important processes and psychological features, such as impulsivity,

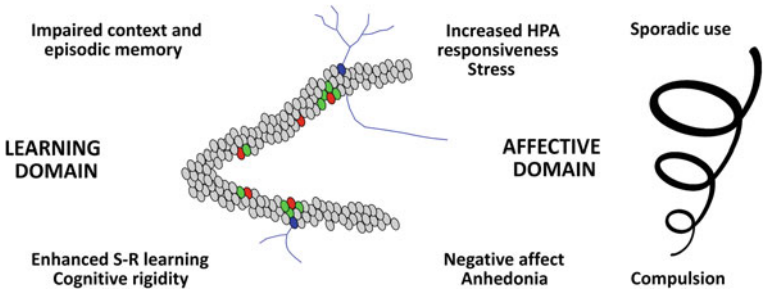

Fig. 1 Deficient hippocampal plasticity impacts negatively on the learning and affective domains contributing to generate a downward spiral loop that leads from sporadic drug use to compulsive drug-taking. Chronic drug exposure reduces hippocampal neurogenesis and affects the learning/cognitive domain, thereby causing deficits in contextual and episodic memory and impaired decision-making, on the one hand, and promoting inflexible patterns of learning and behavior, on the other. Diminished neurogenesis also alters the affective domain, enhancing HPA axis responsiveness and inducing reduced motivation and negative emotion. The *central* diagram depicts the granular layer of the dentate gyrus where stem-like progenitor cells (*red*) give rise to transit amplifying cells (*green*) that mature deep into the granular cell layer (*blue*)

compulsivity, and auto-control, into a biological framework which pictures addiction as a staging process that transits from occasional, reward-based drug usage to recurrent, and compulsive abuse. How neurogenesis in the adult hippocampus influences each of these complex processes is still a matter of speculation, albeit there is little doubt that some important addiction-related behaviors are facilitated by deficient hippocampal plasticity.

One way of gaining insight into the contribution of hippocampal neurogenesis to addictive behavior is by investigating the influence of manipulations that potentiate neurogenesis. An interesting series of studies have examined the influence of environmental enrichment on behaviors specifically related to addiction, such as conditioned place preference (CPP) and relapse to drug-seeking. Enrichment enhances neurogenesis and long-term memory (Bruel-Jungerman et al. 2005), and may play a role in facilitating clearance of outdated memory traces following information transfer to cortical networks (Feng et al. 2001). Conditioned reward is an important psychological correlate of drug administration. In the CPP paradigm, rats subjected to whole brain X-irradiation, exhibited normal conditioning to cocaine, suggesting that depletion of neurogenesis does not block the rewarding effects of cocaine and its association with a given place or context (Brown et al. 2010). However, additional evidence showed that the influence of hippocampal neurogenesis in drug conditioning processes may be more subtle. Long-term exposure to environmental enrichment in mice (e.g., toys, tubes, running wheel) was shown to reverse cocaine-induced CPP and cocaine locomotor sensitization, and also inhibited the expression of cocaine-primed reinstatement in the CPP paradigm (Solinas et al. 2008). Enrichment from weaning to adulthood also blocked in mice heroin-induced CPP, but not heroin-induced stimulation (El Rawas et al. 2009). However, enrichment did not prevent CPP produced by

methamphetamine or methamphetamine-induced neurotoxicity (Thiriet et al. 2011). Although neurogenesis was not evaluated directly in these studies, it is well established that environmental enrichment enhances hippocampal neurogenesis, and therefore such beneficial effects may have accounted for the corrective effects on addiction-related behavior (Solinas et al. 2010). Further work extended these findings to a model of cocaine self-administration. Data revealed that enrichment in rats attenuated stress- and cue-induced reinstatement of drug-seeking, but failed to prevent cocaine-primed reinstatement (Chauvet et al. 2009). Despite the fact that there are significant drug-specific and behavior-specific effects, these findings suggest that environmental enrichment exerts a positive influence on addiction-related behaviors, though further investigation is required to confidently ascribe these effects to enhanced neurogenesis. The introduction of exercise, another intervention associated with potentiation of hippocampal neurogenesis, was shown to accelerate extinction of a cocaine-induced place preference when running occurred after conditioning, enhancing the survival of BrdU+ cells labeled before running began (Mustroph et al. 2011). In the same study, however, exposure to running wheels before conditioning potentiated neurogenesis and delayed extinction of cocaine place preference (Mustroph et al. 2011). The finding that exercise-induced plasticity may strengthen drug-context associations is not consistent with prior observations suggesting preventive effects of enrichment on drug conditioning (Solinas et al. 2008). Additional research is needed to shed light on these seemingly conflicting findings.

Withdrawal from drugs typically elicits dysphoria and negative affect, which are driving forces in the relapse process (Weiss et al. 2001; Koob and Kreek 2007). Neurogenesis in the hippocampus has been implicated in the induction of depressive-like symptomatology and relapse to drug-seeking. Following chronic alcohol exposure, withdrawal from the drug produced depressive-like behavior that was associated with reduced PCNA and double cortin expression in the dentate gyrus (Stevenson et al. 2009). Results in rats exposed to alcohol self-administration and tested for relapse indicated that when withdrawal from alcohol was combined with treatment with the cannabinoid receptor agonist, WIN 55,212-2, an inverse correlation was found between the levels of neurogenesis and alcohol intake during relapse (Alen et al. 2010). Moreover, decreased neurogenesis correlated with enhanced voluntary consumption of alcohol during relapse (Alen et al. 2010). Therefore, pathological adaptations of the neurogenic process in the hippocampus are linked with the appearance of withdrawal-induced negative symptomatology and with enhanced vulnerability to relapse to drug-seeking. Indeed, Noonan et al. (2010) provided a compelling demonstration of how impaired hippocampal neurogenesis may foster addiction-like behaviors. Rats subjected to X-irradiation to ablate hippocampal neurogenesis exhibited a range of abnormalities consistent with the hypothesis that reduced neurogenesis may increase vulnerability to addiction-related behaviors. These abnormalities included increased cocaine intake, enhanced sensitivity to cocaine reinforcement across a wide dose range, heightened motivation to seek cocaine under conditions of progressively increased task demand and

reduced ability to extinguish drug-seeking responses when cocaine was no longer available (Noonan et al. 2010).

6 Conclusions

Despite great advances in defining the role of adult-born hippocampal neurons, and the lack thereof, in the development and maintenance of drug addiction, significant gaps remain in our understanding of such processes. However, a number of firm conclusions can be drawn from the data already available. We have reviewed evidence indicating that contextual and spatial learning, as well as spatial working memory performance, require the integrity of neurogenesis in the adult hippocampus. This evidence suggests that drugs may alter the ability to chunk contextual information composed of spatial and temporal components into coherent memory episodes. This may be particularly true under conditions of elevated anxiety and stress, which often accompany addiction and which may exacerbate the detrimental effects of drugs on neurogenesis. The negative effects of drug exposure on memory may impact on the propensity to take drugs. Indeed, a person with impaired memory may be more likely to make poor decisions and can be more vulnerable to drug abuse.

Individuals who chronically abuse stimulant drugs generally display cognitive deficits, such as mental rigidity. In laboratory animals, this abnormal cognitive attribute might be manifested as an inability to reverse behavioral strategies previously learned, a difficulty in extinction learning or a preference for the use of stimulus-response learning strategies. Both stress and chronic drug exposure affect hippocampal neurogenesis and tend to trigger such cognitive and behavioral deficits in animals and humans. Thus weakened hippocampal neurogenesis, and a subsequently reduced control of the hippocampus over basal ganglia-neocortical systems, may promote inflexible, habitual-like behavior, increase resistance to extinction, and perpetuate drug-seeking actions.

In summary, the analysis presented in this paper suggests that neurogenesis in the adult hippocampus has an influence on several fundamental processes involved in the downward spiraling loop of addiction. Deficient neurogenesis has been linked to a range of components of this spiral including increased sensitivity to drug-induced reward and motivation to seek drug reinforcement, enhanced HPA axis responsiveness, emergence of withdrawal-induced negative states, such as anhedonia, anxiety and depression, wide-ranging memory impairments, and potentiation of inflexible behavior, including impaired extinction learning and enhanced habit learning. The new integrated vision that is beginning to emerge in the addiction field with the incorporation of novel hypotheses and ideas based on ever increasing understanding of adult neurogenesis is still obscured by the scarceness of behavioral data, but a clearer and more complete panorama will surely emerge in the years to come.

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Prolonged Running, not Fluoxetine Treatment, Increases Neurogenesis, but does not Alter Neuropathology, in the 3xTg Mouse Model of Alzheimer's Disease

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Abstract Reductions in adult neurogenesis have been documented in the original 3xTg mouse model of Alzheimer's disease (AD), notably occurring at the same age when spatial memory deficits and amyloid plaque pathology appeared. As this suggested reduced neurogenesis was associated with behavioral deficits, we tested whether activity and pharmacological stimulation could prevent memory deficits and modify neurogenesis and/or neuropathology in the 3xTg model backcrossed to the C57Bl/6 strain. We chronically administered the antidepressant fluoxetine to one group of mice, allowed access to a running wheel in another, and combined both treatments in a third cohort. All treatments lasted for 11 months. The female 3xTg mice failed to exhibit any deficits in spatial learning and memory as measured in the Morris water maze, indicating that when backcrossed to the C57Bl/6 strain, the 3xTg mice lost the behavioral phenotype that was present in the original 3xTg mouse maintained on a hybrid background. Despite this, the backcrossed 3xTg mice expressed prominent intraneuronal amyloid beta (Aβ) levels in the cortex and amygdala, with lower levels in the CA1 area of the hippocampus. In the combined cohort, fluoxetine treatment interfered with exercise and reduced the total distance run. The extent of Aβ neuropathology, the tau accumulations, or BDNF levels, were not altered by prolonged exercise. Thus, neuropathology was present but not paralleled by spatial memory deficits in the backcrossed 3xTg

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Curr Topics Behav Neurosci (2013) 15: 313–340
DOI: 10.1007/7854_2012_237

Published Online: 31 January 2013

mouse model of AD. Prolonged exercise for 11 months did improve the long-term survival of newborn neurons generated during middle-age, whereas fluoxetine had no effect. We further review and discuss the relevant literature in this respect.

Keywords Adult neurogenesis · Alzheimer's disease · Antidepressant · Exercise · Hippocampus

Contents

1 Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive deficits and abundant deposition of amyloid beta (Aβ) plaques and neurofibrillary tangles in selected brain regions. The etiology and progression of the disorder can be studied in transgenic mice that carry one or multiple mutated

copies of the genes that are known to, at least partially, underlie aspects of the disease. The popular 3xTg mouse model for AD was first described in 2003 and contains human mutations in amyloid precursor protein APP, tau, and presenilin 1 that cause it to display both Aβ and tau accumulations. These mice are an attractive model to study AD in view of their robust behavioral and neuropathological phenotype that recapitulates several aspects of the disorder in humans (Oddo et al. 2003a, b). Initial findings from these mice demonstrated that intraneuronal Aβ accumulations not only preceded tau pathology but also correlated with behavioral deficits observed already during early phases of the neuropathology. The increasing levels of intraneuronal Aβ disrupted neuronal integrity and induced cognitive deficits in the mice in the Morris water maze (Billings et al. 2005). The authors further showed that vaccination with an anti-Aβ antibody reversed amyloid accumulation, but not tau neuropathology in these mice, in parallel to the behavioral changes (Billings et al. 2005).

Adult neurogenesis is a form of brain plasticity that refers to the birth of new neurons in adult and aging brains. The extent of neurogenesis is thought to influence vulnerability to accumulating deleterious events during the aging process (Kempermann 2008) and may reflect susceptibility to certain diseases. In the original 3xTg mice, sex and age-dependent deficits in adult neurogenesis were first described in 2008. Female 3xTg mice were shown to have reduced neurogenesis in the dorsal hippocampus starting at 4 months of age and a significant correlation between intraneuronal Aβ and reduced neurogenesis at 12 months of age (Rodriguez et al. 2008). This suggested that a decline in neurogenesis precedes later AD pathology and may correlate with behavioral deficits in the animals. This study was instrumental in stimulating interest in neurogenesis as a therapeutic target to reverse behavioral deficits associated with AD.

Adult neurogenesis is positively regulated by activity in adult rodents (van Praag et al. 1999a, b, 2005; Brown et al. 2003; van der Borght et al. 2009; Kempermann et al. 2010; Marlatt et al. 2010; Lucassen et al. 2010). Running conditions can reverse the decline in hippocampal neurogenesis and improve spatial memory in standard rodent models. For example, aged running mice learn the location of a submerged platform during a spatial memory task similar to young sedentary mice (van Praag et al. 2005).

So far, however, very few studies have investigated effects of stimulating neurogenesis over longer timescales, or addressed its implications for learning and memory late in life. In regard to AD mouse models, wheel running was previously shown to increase neurogenesis in transgenic amyloid precursor protein (APP) mice with the transgenes expressed under the same promoter as that used in the present 3xTG mice (Wolf et al. 2006). Collectively, we predicted that (1) reduction in neurogenesis would relate to behavioral deficits and (2) induction of neurogenesis through activity and pharmacology-dependent mechanisms would rescue these functional deficits. Similar to our recent study in middle-aged wildtype mice (Marlatt et al. 2012), we sought to evaluate the effects of prolonged interventions starting in middle-age in the 3xTg AD mice. Such a design bears relevance for prevention in humans, where AD is often not discovered until late in the course of

the disease when behavioral and neuropathological deficits have manifested already.

Running is a potent stimulus for different stages of the neurogenic process (Kempermann et al. 2010) as well as for spatial memory in mice (Brown et al. 2003; van Praag et al. 1999a, b). It also increases brain-derived neurotrophic factor (BDNF) and angiogenesis (Neeper et al. 1995; van der Borght et al. 2009). As we set out to correlate behavioral changes with neurogenesis, we focused particularly on the survival phase of the newborn cells. Furthermore, as combined running and fluoxetine treatment had previously potentiated the expression of BDNF mRNA in the brain, we expected that application of both running and SSRI treatment would have an aggregate effect greater than either treatment alone (Russo-Neustadt et al. 2000).

We further evaluated the ability of both interventions to impact the extent of neuropathology by measuring Aβ and tau pathology in the 3xTg mice. A relatively novel aspect of the biochemistry and neuropathology of Aβ is the accumulation of intraneuronal Aβ as observed in human brain and several studies now suggest it is the intraneuronal Aβ that forms one of the earliest pathological events in AD (Gouras et al. 2000; Wirths et al. 2004; Christensen et al. 2009, 2010). As synaptic pathology correlates better with cognitive dysfunction than the accumulation of amyloid plaques (Terry et al. 1991), many studies of Aβ seek to establish a mechanistic link between Aβ and the physical damage to synapses and neurons (Bayer and Wirths 2010; Gouras et al. 2010). We here show that despite the loss of the behavioral phenotype in these backcrossed animals, the 3xTg mice express prominent levels of intraneuronal Aβ in the cortex and amygdala, with lower levels in the CA1 area. Fluoxetine seemed to reduce Aβ accumulations. Although the overall extent of neuropathology and BDNF levels remained unaltered, prolonged exercise significantly increased neurogenesis. Possible explanations and similar correlations in related models will be discussed.

2 Materials and Methods

2.1 Mice

3xTg mice were first generated by Dr. LaFerla (University of California, Irvine, USA) and acquired by the National Institute on Aging (NIA, Baltimore, MD, USA). The 3xTg mice, harboring PS1M146 V, APPswe, and tauP301L transgenes were first backcrossed to the C57Bl6 strain for seven generations. All mice were maintained on a standard NIH-07 diet (Harlan-Tekland, Indianapolis, IN) with free access to water during a 12-h light/12-h dark cycle. Female mice were group housed until the start of the experiment at 9 months of age and then singly housed for the duration of the experiment. Female 3xTg mice ($n = 10$ per group) were randomly assigned to control (Con), fluoxetine (Flu), running (Run), and

synergistic treatment with fluoxetine and running (FluxRun). Running mice (Run and FluxRun groups) were housed with a running wheel and distance run was recorded daily (Clocklab, Coulborn Instruments, Whitehall, PA).

Two cohorts of animals were used to evaluate acute and chronic responses to the described conditions. Cohort 1: Animals were chronically treated with the conditions listed above for 10 months, with behavioral testing at 1 and 10-month time points. Mice were euthanized on day 333, 11 months after the start of the study at 20 months of age. Cohort 2: Animals were treated acutely for 1-month ($n = 3$–4 per group), to determine the acute effects of treatment on the survival of new neurons labeled in the DG. At the end of each study, animals were deeply anesthetized by isoflurane inhalation and perfused with phosphate buffered saline. Animals were decapitated and brains were immediately removed. The right hemisphere was placed in 4 % paraformaldehyde for 48 h, followed by equilibration in 30 % sucrose. Tissue was sectioned coronally (40 μm) on a freezing microtome (Thermo-Fisher) and stored at −20 °C in cryoprotectant solution. The left hemisphere was dissected and frozen on dry ice for biochemical analysis of BDNF. All animal procedures were done in accordance and were approved by the National Institute of Health Animal Care and Use Committee.

2.2 Administration of Fluoxetine in Drinking Water and Measurement of Running Distance

Fluoxetine was dissolved in drinking water and replaced every 7 days. A pilot study established that running mice drink comparable amounts of water as sedentary controls. Fluoxetine is soluble in water up to a concentration of 4 mg/ml; in this study, we dissolved fluoxetine at 0.12 mg/ml such that oral dosing was 18 mg/kg/day (based on pilot data on water consumption).

All of the mice were individually housed ($n = 10$ per group) at the start of the experiment with two groups assigned to running wheel cages. The average distance run per day was 2.2 ± 0.6 km for runners and 1.1 ± 0.5 km for runners that were additionally treated with fluoxetine (see Table 1), but this was not statistically significant.

2.3 Behavior: Morris Water Maze

Mice were trained in the Morris water maze (Morris 1984), to find a platform hidden 5 mm below the surface of a pool (1.40-m dia.) that was filled with water made opaque with white nontoxic paint. Starting points were changed daily for each trial. A trial lasted either until the mouse had found the platform or for a maximum of 60 s. Mice rested on the platform for 10 s after each trial. Mice were

Table 1 Total distance range and average for Flu and FluxRun groups

Groups	Days	Range: Total distance (km)	Average distance (km)	Stdev. (km)
Run	1–28	5.4–123.0	54.2	51.4
Fluxrun	1–28	2.1–120.4	40.3	44.3
Run	1–333	231–1367	738	461
Fluxrun	1–333	28–1188	367	420

Data collected from the running wheels reflects that both Run and FluxRun groups had a high degree of variation. While exercise significantly increased the distance ran FluxRun animals showed a trend for running less distance on average than the Run cohort alone, however the means were not significantly different (Student's 2-tailed *t*-test, 28 days $p = 0.61$, 333 days $p = 0.09$)

trained with four trials per day over 6 days. Upon completion of training, the platform was removed for 60-s probe trials; probe trial were held 4 h and 24 h after the last training session. Latency to reach the platform was recorded during the first 6 days and the time in each quadrant was measured during the probe trials (Anymaze, Stoelting Co., USA).

2.4 Behavior: Rotarod Performance

The rotarod test was used to assess sensorimotor coordination and motor performance at 10 and 20 months of age. The total number of falls were measured during three trials of 5 min each using a program with constant acceleration to 25 rpm (Med Associates, VT).

2.5 Bromo-deoxy-uridine Immunohistochemistry and Cell Counts

In order to analyze newborn cells, Bromo-deoxy-uridine (BrdU) (50 mg/kg) was injected i.p. for the first 10 days for each cohort of animals. A one-in-six series of free-floating sections (40 μm) was washed in PBS and pre-incubated with 0.6 % H_2O_2 for 30 min. After rinsing, the sections were incubated in 2 N HCl at 37 °C for 30 min to denature DNA and then neutralized in 0.1 M borate buffer at room temperature (RT). After thorough washing, the sections were blocked with PBS ++ (3 % donkey serum-0.05 M PBS, 0.5 % Triton-X 100) for 30 min at RT and incubated with rat anti-BrdU (1:200 in PBS, Accurate Chemical Westbury NY) overnight at 4 °C. Thereafter, the sections were washed and immersed in biotin-SP-conjugated donkey anti-rat IgG (1:250, Jackson Immuno Research, West Grove, PA) followed by 2 h in ABC reagent (1:800, Vestastain Elite; Vector Laboratories, Burlingame, CA). The sections were then incubated with the

substrate 3, 3′-Diaminobenzidine (D4418, Sigma, St. Louis, MO) for 5 min to visualize the cells that had incorporated BrdU. BrdU-positive cells were counted in a one-in-six series of sections (240 μm apart) through a 20X objective (Olympus, BX51) throughout six hippocampal sections per animal starting at approximately Bregma −1.46.

The volume of the dentate gyrus (DG) for each group of animals was determined by DAPI staining a 1:6 series of sections and outlining the granular cell layer (GCL) and subgranular zone (SGZ) on a microscope equipped with Stereo investigator software (Microbrightfield, Burlington, VT). Seven sections were outlined making boundary contour tracings to determine the area of the DG at each level. Area values were analyzed with the Cavalieri method to determine hippocampal volume.

2.6 Double Immunofluorescence for Cell Fate Analysis

Free-floating sections (1:6 series) were simultaneously incubated with primary antibodies against BrdU (1:100 Accurate Chemical Westbury NY) and the neuronal marker NeuN (1:100 Millipore, Billerica, MA) after the denaturation, neutralization, washing, and blocking steps described above. Antibodies were diluted in PBS ++ and then sections were incubated for 48 h at 4 °C. After rinses with PBS and blocking in PBS ++, sections were co-incubated with donkey anti-rat Alexa Fluor 488 (1:250, Molecular Probes, Carlsbad, CA) and donkey anti-mouse Alexa Fluor 568 dyes (1:250, Jackson Immuno Research, West Grove, PA) for 2 h at RT. A laser-scanning microscope was used to identify cells positive for both BrdU and NeuN markers. Fluorescent signals were imaged with a Zeiss LSM 510 confocal laser-scanning microscope and confocal and z-stacked images were used in coordination to determine the percentage of BrdU-positive cells with a neuronal phenotype by co-labeling expression of BrdU with NeuN.

2.7 Aβ Immunohistochemistry and Densitometry

The extent of Aβ immunoreactivity was determined by using a technique documented previously that utilizes formic acid (FA)-based antigen retrieval (Christensen et al. 2009). This procedure was modified to accommodate floating sections cut at 40 μm sections. These sections were washed and mounted on Superfrost slides. Antigen retrieval was achieved by boiling sections in 0.01 M citrate buffer (pH 6.0) followed by a 3-min incubation with 88 % FA. The primary Ab was directed against N-terminal Aβ peptides (1:250 IBL Japan #18584). Sections were developed using biotinylated secondary Ab, Goat anti-Rabbit 1:200, and Vector Labs ABC (Vector Labs, Burlingame, CA USA). Images, taken at total 100x magnification, were collected on an Olympus BX-51 microscope equipped with a

DP-50 camera (Olympus, Hamburg, Germany). Images were converted into 8-bit grayscale images with NIH Image J (v. 1.61) and then converted into binary positive/negative images by using a threshold limit held constant for all images in a given brain region. Percent area fraction was determined through the use of a macro in Image J.

2.8 Tau Immunohistochemistry and Densitometry

As protein tau is aberrantly hyperphosphorylated at serine and threonine residues in AD, we used the monoclonal Ab AT8 that recognizes tau protein phosphorylated at both serine 202 and threonine 205 (Goedert et al. 1995). Tau accumulations can occur in the absence of Aβ and serve as hallmarks for neurodegenerative tauopathies (Lee et al. 2001; Goedert et al. 1992).

AT8, a mouse monoclonal Ab, was blocked with goat anti-mouse FAb fragments to bind endogenous mouse antigens (1:200 goat anti-mouse FAb fragments diluted in 2 % NGS, 0.4 % triton, 0.1 M PBS). After subsequent thorough washing (6 × 10 min in PBS), sections were incubated with 1:1000 AT8 for 1 h at RT and 4 °C overnight. The following day sections were washed and incubated with biotinylated sheep anti-mouse (1:200). Avidin–Biotin complex was applied at the lab-tested concentration (1:800) and sections were developed with diaminobenzidine (DAB) according to standard procedures.

Densitometry was used to measure Aβ deposition as labeled by immunohistochemistry with anti-Aβ antibody (IBL #18584) recognizing the N-terminus of Aβ present in both intraneuronal and extracellular Aβ. Densitometry employed a thresholding technique that captures intraneuronal and extracellular Aβ deposition information indiscriminately and simultaneously. For each brain subregion, available images were saved as an image sequence and a threshold was determined that identified specific staining while omitting background staining in the sections. The image sequence was then evaluated for percent area fraction using three threshold values, namely the originally determined threshold value in addition to one higher and one lower. Under no circumstances did changing the threshold value, used to determine the percent area fraction, change the outcome of the statistical comparison between groups.

2.9 BDNF Western Blot

To measure mature BDNF peptide levels, hippocampal tissue was homogenized in 400 µl of the 1X RIPA buffer containing protease inhibitors (Complete Mini, Roche Diagnostics) using pestles and microtubes (ISC BioExpress) and then sonicated with four pulses of 10 s at scale 4 (Ultrasonic Processor, Model GE70) at RT. The lyzed samples were centrifuged at RT for 10 min and the supernatants

were transferred to fresh tubes. The lysates were reduced with 100 mM DTT at 70 °C for 1 h to break the strong disulfide bonds of BDNF. The protein concentrations were measured using Bradford method (Bio-Rad). The samples were diluted to final concentration of 3 μg/μl with the lysis buffer and 4X LDS NuPAGE sample buffer (Invitrogen). Before electrophoresis, the samples were heated at 90 °C for 5 min, rapidly cooled on ice for 1 min and then equilibrated to RT for 10 min. Equal amounts of 15 μg of the proteins were loaded onto 4–12 % gradient NuPAGE neutral polyacrylamide gel. The electrophoresis was carried out in 1X MES buffer and the proteins in the gel were transferred to Immobilon-FL membrane (Millipore) using NuPAGE transfer buffer according to the manufacture's protocol (Invitrogen). The polyclonal rabbit BDNF antibody (Santa Cruz Biotechnology, Inc.) and an infrared-labeled goat against rabbit secondary antibody (Li-Cor Biosciences) were used for immunostaining according to Li-Cor's protocol. The specificity of BDNF antibody staining was confirmed by co-migration with the reduced human recombinant BDNF (0.1 mg) monomer (Neuromics).

2.10 Statistical Analysis

All statistical analyses were carried out using GraphPad Prism. For Morris water maze latency, one-way analysis of variance (ANOVA) with repeated measures was performed followed by Bonferroni posthoc tests for individual days. For the time in quadrants, a one-way ANOVA was performed on each group followed by Bonferroni post hoc tests. For comparisons of two groups, Student's t test was used to determine if means were significantly different and assign statistical significance.

3 Results

3.1 Motor Impairment in Fluoxetine-Treated Animals

Running distances in the Run and FluxRun cohort were collected during the study. Surprisingly, a high degree of variability was present in the middle-aged mice for running distance (Table 1). When assessing motor coordination and performance using the rotarod, motor impairment was evident at 20 months of age in the 3xTg mice treated with fluoxetine compared to all our groups. Mice treated with fluoxetine displayed a significantly higher frequency of falls particularly in trials 1 and 3 (Fig. 1, One-way ANOVA $p < 0.05$).

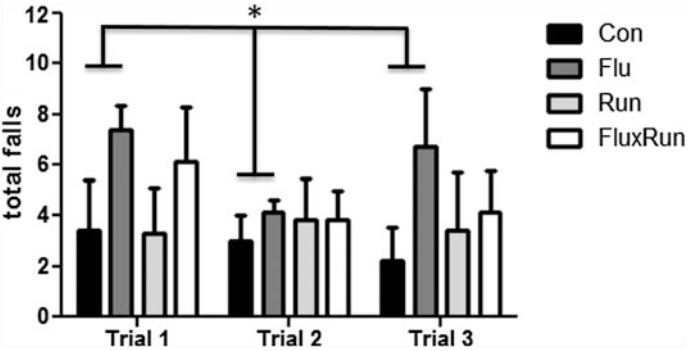

Fig. 1 Total falls in the rotarod test at 20 months of age. During a program of constant acceleration on the rotarod rod, 3xTg mice given fluoxetine showed a higher total number of falls when measured across trials. All three trials were conducted in succession on the same day with approximately 1 h between trials (repeated measures ANOVA $F(3,54) = 5.82$, $p < 0.05$)

3.2 Acute and Chronic Running Stimulate BrdU+ Cell Survival and Adult Neurogenesis

To label dividing neural progenitor cells, the thymidine analog BrdU was injected for the first 10 days. To measure the short-term effects of running and fluoxetine, a separate cohort of animals was used to quantify BrdU+ cell survival 1 month after injection when 3xTg mice were 10 months of age. BrdU-labeled cells were counted in the SGZ and granule cell layer. At this time, there was a high amount of variability between groups and mean cell survival was not significantly different between groups (Fig. 2a).

To measure long-term effects of running, fluoxetine, and synergistic treatment, BrdU+ cell survival was also measured after 10 months of treatment when the animals were 19 months of age. A significant difference was found between treatment groups, specifically that running and combined fluoxetine treatment had elevated the number of surviving BrdU+ cells, albeit significantly less so in the FluxRUN as compared to the Run group (Fig. 2b). However, neither group showed a significant correlation between running distances and BrdU+ survival (data not shown: groups combined, Pearson $r = 0.23$, $p = 0.2125$; Run only, Pearson $r = 0.14$, $p = 0.38$; FluxRun, Pearson $r = 0.28$, $p = 0.27$).

3.3 Neuronal Differentiation Is Unaffected by Treatments

To investigate the ability of running, fluoxetine, and synergistic treatment to influence adult born cell fate and neurogenesis, confocal imaging was used to also identify BrdU +/NeuN+ cells. BrdU in this paradigm identifies cells born during

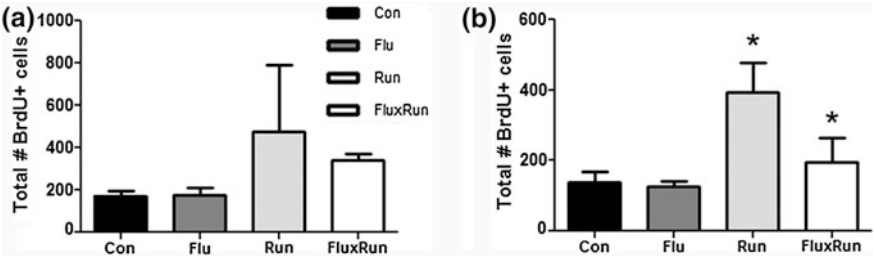

Fig. 2 BrdU + cell survival 1 and 11 months after beginning treatment. **a** 1 month after treatment with fluoxetine (*Flu*), exposure to running (*Run*), or combined therapy (*FluxRun*), none of the treatment groups showed significant elevations in BrdU+ cell survival compared to controls (*Con*) (One-way ANOVA $F(3, 9) = 0.97$, $p = 0.44$). **b** 11 months after starting treatment, significant increases in BrdU+ cell survival were seen for both *Run* and *FluxRun* groups compared to nonrunning controls. (One-way ANOVA $F(3,23) = 5.27$, $p < 0.01$)

the first 10 days of the experiment, while NeuN identifies mature neurons. Co-imaging of these markers across cohorts showed there were no significant differences in the percentage of cells labeled with both markers (control: 65 ± 11 %, Flu 59 ± 7 %, Run: 77 ± 7 %, and FluxRun 48 ± 12 %) (Fig. 3).

The volume of the DG for each group of animals was determined by DAPI staining in a 1:6 series of sections. Area values were used by the Cavalieri method to determine hippocampal volume. No significant differences in total area were found between groups (One-way ANOVA $p = 0.86$, $F = 0.25$).

3.4 Aged 3xTg Mice Do Not Exhibit Impairments in Learning or Memory

To assess spatial learning and memory, mice were tested in the Morris water maze. Surprisingly, there were no observable differences between groups after 1 month of treatment (Fig. 4a). All animals in the study learned the task to criterion within 6 days, with both groups remembering equally well the location of the platform in a probe test 24 h after the last training session. After 11 months of running, at 20 months of age, mice were trained to a different platform location. There was no difference between groups in acquisition of the task over 6 days of training or in the probe tests. During the 6- and 24-h probe tasks, mice in Con, Run, and FluxRun had a preference for the target quadrant (Fig. 4c, d). Mice treated with fluoxetine, did not show this preference. No differences in swim speed or path length were detected between groups (data not shown).

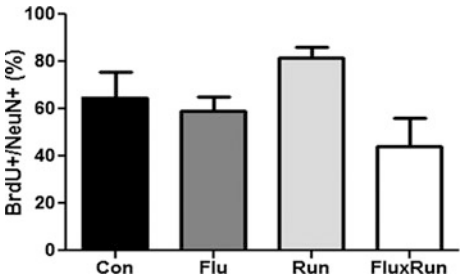

Fig. 3 Percentage of BrdU + cells expressing the mature neuronal marker NeuN. None of the treatment groups showed significant differences in the percentage of BrdU + newborn cells expressing NeuN as marker for mature CNS neurons (One-way ANOVA $F(3,19) = 2.27$, $p = 0.11$)

3.5 Mature BDNF Protein Levels in the Hippocampus

Mature BDNF was measured by Western blot; there was no significant difference in BDNF expression between the groups (Fig. 5).

3.6 Aβ Expression in the 3xTg Mouse

Given the important role of intraneuronal Aβ in the pathogenesis, we used immunocytochemistry to detect intraneuronal Aβ. This technique also identifies extracellular Aβ plaques, but not beta C-terminal fragments (β-CTFs). In sections of the rostral hippocampus, intraneuronal Aβ was observed primarily in the cortex and amygdala (Amy) (Figs. 6a, b, and 7a–h). In contrast to these areas, extracellular Aβ plaques were predominantly found in the CA1 region and Subiculum (Figs. 6c, d, and 7i–p). In order to make appropriate comparisons, these structures were quantified by densitometry across three separate sections that were standardized to specific rostral-caudal orientations (representative levels Fig. 6). A 1:6 series of tissue sections was used to assess levels of neuropathology, such that neighboring sections are 240 μm apart. If three consecutive sections were not available for quantification within an anatomical region, within the proper rostral-caudal orientation (amygdala and cortex $-1.46 \rightarrow -2.30$ mm, CA1 $-1.94 \rightarrow 2.78$ mm, subiculum $-2.46 \rightarrow -3.30$ mm), the animal was not included for analysis.

In the 3xTg mice, most amyloid pathology was present in the subiculum, amygdala, and hippocampal CA1 areas, whereas the dentate gyrus, e.g., was largely devoid of amyloid.

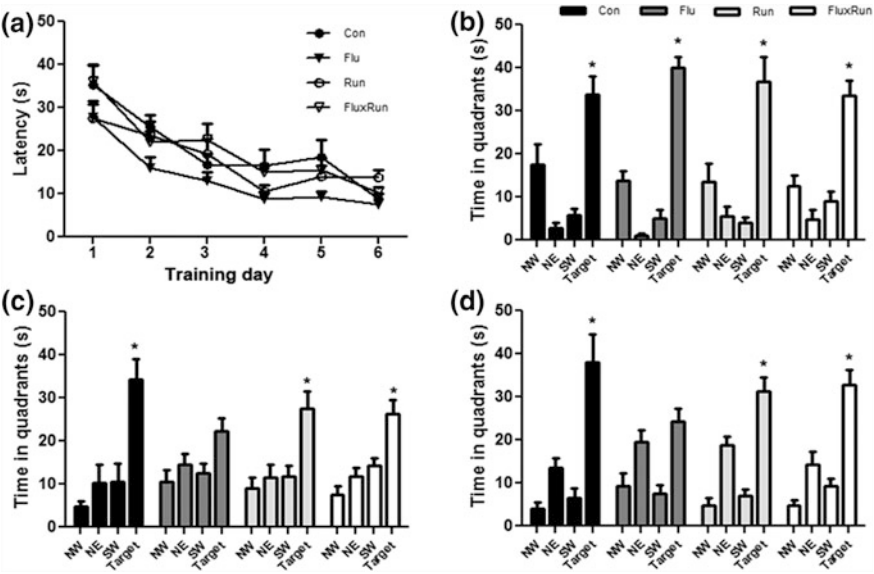

Fig. 4 3xTg mice show no impairment in learning or memory during testing in the MWM at 10 or 20 months of age **a** 1 month data reflects that all groups learned the MWM to criterion (latency to platform <20 s) (repeated measures ANOVA $F(3,198) = 1.97$, $p = 0.16$) and **b** all groups at the 1 month timepoint, remembered the location of the platform 24 h after their last training session (*Con* $F(3,27) = 13.44$, *Flu* $F(3,27) = 54.91$, *Run* $F(3,24) = 11.60$, *FluxRun* $F(3,27) = 17.09$, $P < 0.001$) Bonferroni post test $p < 0.05$ target versus each quandrant **c** 10 months after starting the experiment, 3xTg mice were re-trained and *Con*, *Run*, and *FluxRun* showed significant preferences for the target quadrant during 4 h probe trials (*Con* $F(3,12) = 9.00$, *Run* $F(3,21) = 6.00$, *FluxRun* $F(3,18) = 9.34$, $P < 0.01$) Bonferroni post-test $p < 0.05$ target versus each quandrant, *Flu* $F(3,21) = 2.94$, $p = 0.06$) **d** 24 h probe trials (*Con* $F(3,12) = 13.14$, *Run* $F(3,21) = 21.50$, *FluxRun* $F(3,18) = 22.81$, $P < 0.01$) Bonferroni post-test $p < 0.05$ target versus each quadrant. *Flu* did not show a significant preference for the target quadrant

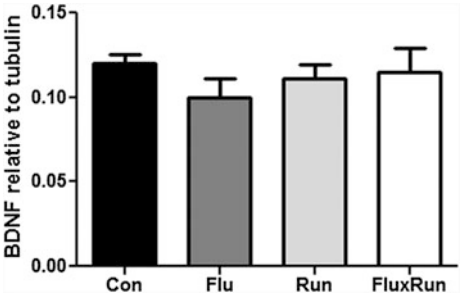

Fig. 5 Mature BDNF protein in the hippocampus. No significant differences were observed in the amount of soluble BDNF across cohorts. BDNF protein, as measured by Western blot, was standardized to the housekeeping gene, beta-tubulin (One-way ANOVA $F(3,23) = 0.56$, $p = 0.64$)

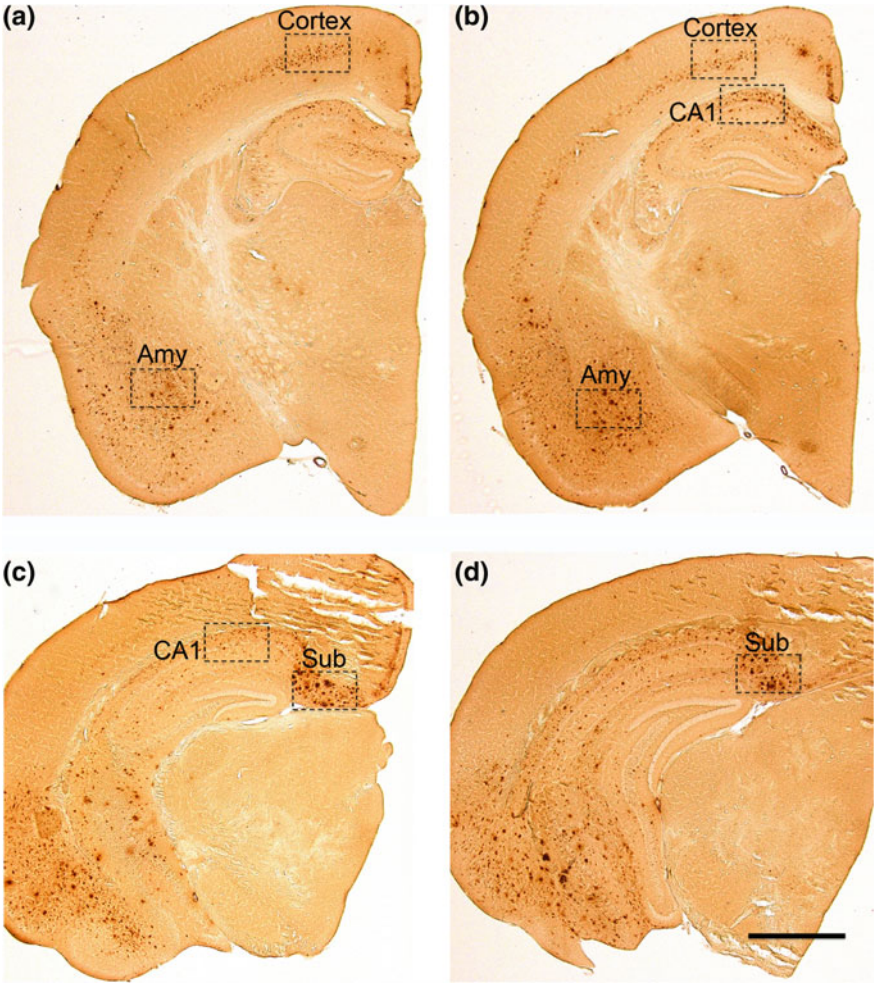

Fig. 6 Anatomical overview and regions of interest used for quantification of Aβ pathology in 20 month-old female 3xTg mice. **a** Cortical and amygdala Aβ pathology were quantified at approximately Bregma −1.46 mm. **b** Cortical, amygdala and CA1 pathology were quantified at −1.94, while quantification of the subiculum continued through −2.46, **c** staining of the subiculum was quantified in the caudal hippocampus, here at −2.70 mm, but as far back as −3.30 mm. scale bar = 600 μm

3.7 Hyperphosphorylated Tau in the Caudal Hippocampus

Tau pathology, known to correlate well with cognitive decline and hence an important measure for neurodegeneration in AD models, was quantified following immunocytochemistry for AT8, a well-known pathological tau epitope. AT8 expression was particularly prominent in the CA1 region in the 3xTg mice at this

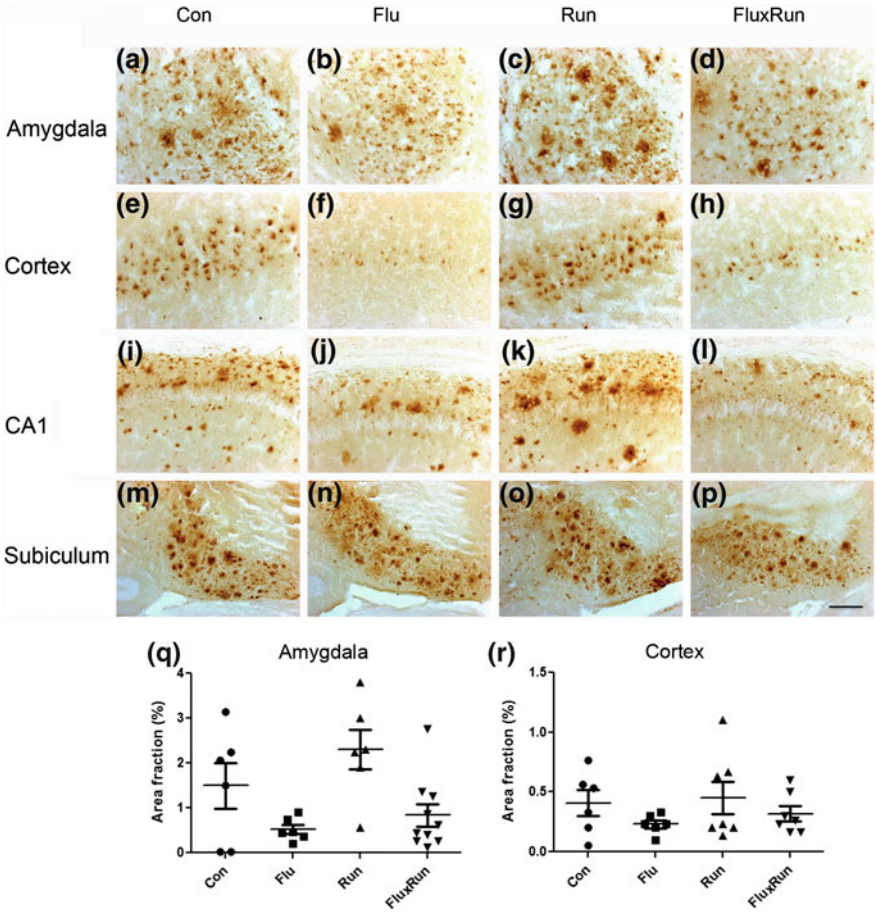

Fig. 7 Intraneuronal Aβ and Aβ plaque load in selected subregions of the 3xTg mouse brain. **a–d** Examples of Aβ deposition in the amygdala of 3xTg mice, showing intraneuronal Aβ and extracellular Aβ deposits across cohorts. **e–h** Intraneuronal Aβ was found in the cortex of all cohorts, with little evidence of extracellular deposits. **i–j** In the CA1 region, the majority of detected Aβ was present in extracellular plaques, but no neuronal profiles were seen when compared to the cortex. **m–p** A dense staining of extracellular plaques was predominantly found in the subiculum across cohorts (scale bar = 100 μm). **q** Densitometry of the amygdala across cohorts: *Flu* and *FluxRun* groups had lower area fractions scores compared to *Run*, but not to the *Con* groups. (One-way ANOVA $F(3,24) = 4.79$, $p < 0.05$) Bonferroni post-test *Flu* versus *Run* $p < 0.05$, *FluxRun* versus *Run* $p < 0.05$). **r** In the cortex, fluoxetine appears to reduce Aβ accumulations, however, these differences were not statistically significant (One-way ANOVA $F(3,22) = 1.00$, $p = 0.41$)

age, with some minor expression extending into the subiculum but hardly, if any expression in other brain regions (Fig. 8).

4 Discussion

4.1 Discussion of the Present Data on the 3xTg Mice

Our current data demonstrates that running, when started in middle-age, increased survival of newborn neurons in 3xTg mice studied at old age. Cells identified by BrdU-labeling in this experiment survived for 11 months, indicating that neurons generated in middle-age are indeed maintained through old age. As we identified newborn cell survival both after 1 and 11 months of running and fluoxetine treatment, it is clear that only a minor loss of the labeled newborn neurons occurred during the 10-month period in between; in control 3xTg mice, an average of 173 labeled cells were observed after 1 month and an average of 149 surviving cells per DG after 11 months (Fig. 2a, b). Hence, the selection process for new cells appears to occur primarily during their first month of life. Further comparing the amount of neurogenesis observed here to that documented in our previous study in wildtype mice (Marlatt et al. 2012), shows that the 3xTg mice have lower neurogenesis levels per se compared to the C57Bl6 mice parental strain.

In 20-month-old mice, 11 months of running and synergistic treatment both increased survival of new cells. Interestingly, this occurred in fluoxetine treated runners despite evidence that fluoxetine impairs motor performance. Indeed, the increase in BrdU labeling was less in the FluxRun than Run animals. Data from the wheel runners indicate a trend for a reduced total distance in animals given fluoxetine in their drinking water. Previously, fluoxetine has been shown to significantly reduce running wheel activity also during acute treatment (Weber et al. 2009). While confirming evidence for reductions in running wheel activity during short time periods, our study further shows that long-term drug treatment impairs motor coordination in 3xTg mice. Prior to starting this experiment, there was evidence that fluoxetine could increase activity (Brocco et al. 2002; Prinssen et al. 2006), but currently it is unknown if this occurs in a dose-dependent manner, or how exactly fluoxetine influences activity of mice.

In the literature, benefits have further been reported with the use of antidepressants in 3xTg mice. While neurogenesis may be altered in human depression and can be responsive to antidepressive treatment (Lucassen et al. 2010a; Boldrini et al., 2009), the effects of several antidepressant drugs on neurogenesis depend on the age of the animal, its early-life history and the mouse strain under study (Couillard-Despres and 2009; Navailles 2008). Indeed, in the present study, similar to our findings in C57Bl/6 mice (Marlatt et al. 2010) fluoxetine did not increase neurogenesis in 3xTG mice. Amitriptyline when given for 4 months was shown to increase neurogenesis, to improve performance in the MWM, and to increase

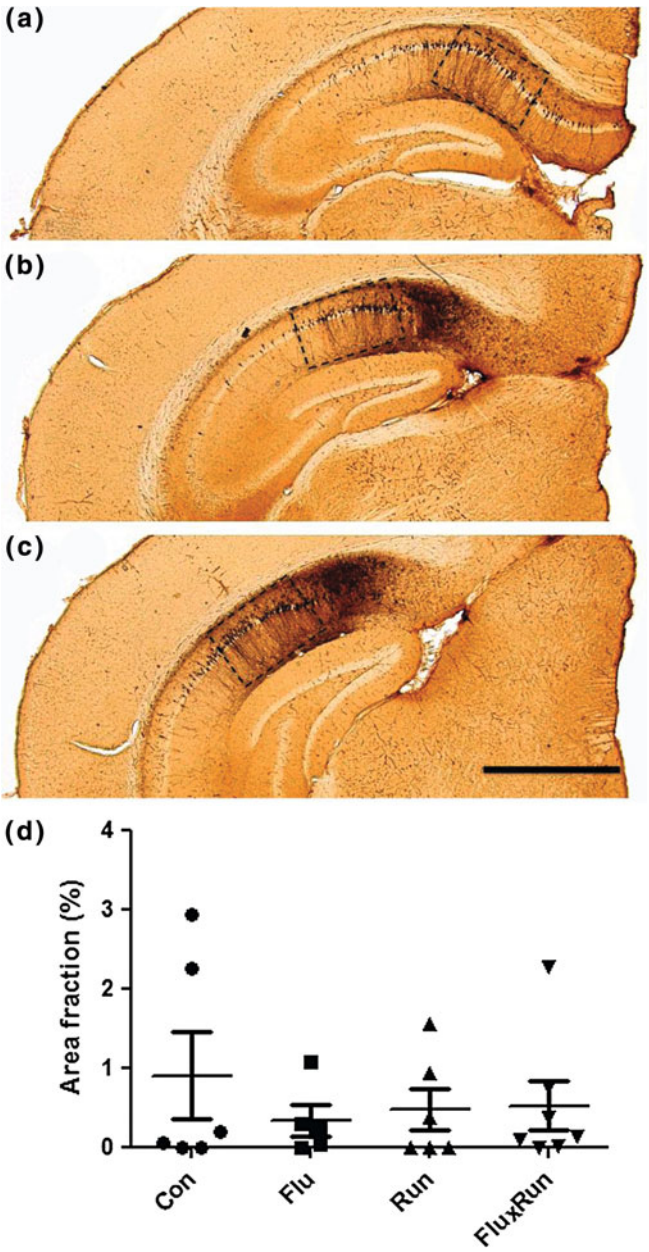

Fig. 8 AT8 tau pathology in the CA1 region of the 3xTg mouse. **a–c** Neurons of the CA1 regions showed robust staining for hyperphorlated tau protein as identified by AT8 immunostaining. While being photographed sections were positioned to exclusively frame CA1. Quantification was carried out on sections beginning at −1.94 mm Bregma and continuing through approximately −3.16 mm Bregma, scale bar = 600 μm. **d** None of the interventions showed an ability to reduce pathological hyperphosphorylation (ANOVA $F(3,22) = 0.58$, $p = 0.61$)

BDNF protein levels in male 3xTg mice (Chadwick et al. 2011). Paradoxically, however, this study also showed that amitriptyline increased Aβ deposition in the brain. This surprising finding highlights that while antidepressants, such as fluoxetine, do not have a known direct pharmacology for APP and the generation of Aβ peptide, these drugs can nonetheless impact its clearance and deposition and there may be secondary mechanisms capable of influencing Aβ accumulation in the brain. Earlier work had established that paroxetine, another antidepressant from the SSRI class, reduced expression of APP by binding a promoter region in the 5′UTR (Tucker et al. 2005) while this drug also showed cognitive benefits in the Morris Water Maze (Nelson et al. 2007). Together, this indicates that antidepressants can exert indirect effects on Aβ plaque deposition in the 3xTg model, each with unique mechanisms of action. Whether these drugs can have a meaningful impact on Aβ deposition in humans remains to be determined.

4.2 Behavioral Phenotype of 3xTg Mice

As a delayed behavioral phenotype had already been observed in younger backcrossed 3xTg mice, we started the experiment when our animals were 9 months of age. In a study of 12-month-old male 3xTg mice increased anxiety was reported during a 6-week trial of psychosocial stress (Rothman et al. 2012). A comparable study utilizing male 3xTg mice, starting at 14 months of age, reported behavioral deficits at 18 months of age (Chadwick et al. 2011). These reports on the backcrossed strains demonstrated a significant delay in the development of their behavioral phenotype when compared to the original strain, where cognitive deficits were documented as early as at 6 months of age in both male and female 3xTg mice (Billings et al. 2005). Hence, for future behavioral studies with the 3xTg mice, the original strain would be preferred as also discussed in more detail below.

Testing spatial learning and memory in the MWM is known to represent learning under stress and produces robust elevations in corticosterone release, the predominant glucocorticoid hormone in rodents (Schaaf et al. 1999). Occupancy of glucocorticoid receptors (GR) is known to play a critical role in determining performance in the MWM (Oitzl et al. 1998). Interestingly, female 3xTg mice show elevated corticosterone levels after 5 days of MWM training, a sexual dimorphism not present in male 3xTg mice (Clinton et al. 2007). In our present MWM experiments, corticosterone levels were not quantified but future studies with the 3xTg model should ideally measure corticosterone concentrations in both sexes. Also, additional spatial tasks, such as the Barnes maze or the object recognition test, that generally induce a smaller or no stress response, should be used in parallel to test the contribution of a stress response to spatial performance in these mice.

Wheel running in 3xTg mice has been shown to attenuate cognitive deficits. Interestingly, these were only found in the female running mice (Pietropaolo et al.

2008). Also, other behavioral changes have been observed. Chronic exercise could prevent deterioration of anxiety and startle responses in the 3xTg mice (García-Mesa et al. 2011). Outside of the traditional markers of AD neuropathology, 6 months of wheel running was further shown to reduce markers of oxidative stress in the brains of 7-month-old 3xTg mice and to protect these mice against an age-related loss of synaptic integrity (García-Mesa et al. 2011). A separate study showed that voluntary running increased neurogenesis when evaluated at 9 months of age; running, however, did not change the fate of the newborn cell phenotypes, which agrees with our current results in the same 3xTg mice (Rodriguez et al. 2011) but not with our previous study in younger C57Bl6 mice (Marlatt et al. 2010).

4.3 Backcrossed Versus Orginal 3x Tg Mice

Although the old, backcrossed 3xTg mice lack a strong behavioral phenotype, the animals used in the present experiments were systematically genotyped and did exhibit both Aβ and tau accumulations in their brains. This indicates that AD pathology can occur in absence of an appreciable behavioral phenotype. Most likely other genes from the C57 background have contributed here, that could have influenced the rate of decline, or even have overruled possible deleterious effects, if any, of the AD pathology present in these mice. An alternative option is that more abundant expression patterns might be needed before behavioral deficits can develop, while it is also possible that the presence of plaque and/or tau pathology in these key brain regions does not necessarily predict behavioral deficits and, as in other models, might follow, rather than precede, behavioral deficits in time (Oddo et al. 2003b; van Dooren et al. 2005; Götz and Ittner 2008). Identifying the exact factors responsible for these effects in the backcrossed strain of 3xTg mice and why they are different from the original strain, would require additional experiments.

One option would be to identify quantitative gene expression changes, by microarray and quantitative PCR, for each region of the brain. Identified candidates from the screening could be prioritized based on known protein structures and functions. Ideally, Western blotting data combined with immunohistochemistry could identify subcellular changes in distribution or cell type. Further analysis of soluble and insoluble fractions of Aβ peptide by ELISA might shed light on the proteolytic cleavage of APP. Further experiments would also be needed to identify possible changes in Aβ oligomers and determine if trafficking and/or clearance of these proteins is altered in the backcrossed strain.

At this time we can only speculate that backcrossing the 3xTg mouse may have led to a loss of intraneuronal Aβ expression in the CA1 subregion. This is difficult to address because no mice were available from the original strain, of the same age, and stained with the same antibody. Moreover, we used an antibody directed against N-terminal Aβ (IBL #18584). Previously, in the hybrid 3xTg strain at ages of 2, 14, and 20 months, a monoclonal antibody directed at amino acids 1–5 of the

N-terminal Aβ variant (3D6, Elan, South San Francisco, CA, USA) has been used to visualize Aβ deposits (Cai et al. 2012). These authors found that 3D6 labels Aβ deposits across the cortex and pyramidal layer in 2-month-old animals, where it appears as perisomatic puncta or granules. In 14-month-old animals, intraneuronal puncta labeling was also seen in the CA1. In 20-month-old transgenics, puncta are evident in the CA1, where 3D6 labels around the neuronal somata, however, this is less dense than the intraneuronal staining seen in the cortex (Cai et al. 2012) (Fig. 9, panels a, b). A conformation-specific antibody, which identifies fibrillar oligomers (Kayed et al. 2007), has also been used to identify intraneuronal CA1 staining in the 3xTg mice, aged 18 months, that were maintained on the hybrid background (Wirths et al. 2011) (Fig. 9, panels e, f). By comparing the staining patterns between hybrid and backcrossed 3xTg mice for cortical and hippocampal subregions, this limited evidence indicates that a loss of intraneuronal Aβ in the hippocampus may contribute to, or possibly, explain the loss of the present behavioral phenotype.

In further discussions of the 3xTg mice, others have also observed significant problems in retrieving the originally described phenotype after backcrossing (Dr. E. Hol, Amsterdam, personal communication). The 3xTg mice are now widely available from commercial vendors, namely the Jackson laboratory, and several investigators that have received mice generated from frozen embryos found that the phenotype of these animals were significantly delayed (personal communication Dr. Jorge Palop). Thus, for future studies with 3xTg mice, ideally, the original strain would be preferred.

4.4 General Discussion of Neurogenesis in Relation to Alzheimer Pathology

While synaptic plasticity is thought to be the main structural change corresponding to rapid changes in cognitive function, the addition of new neurons to an existing circuit through adult neurogenesis represents a unique form of structural plasticity for the longer term. In young rats, it has been estimated that approximately 9000 new cells per day are generated in the entire hippocampus, and a considerable proportion of these cells die within several days. While the number of new neurons incorporated into the DG may be quite low, particularly during aging, this ongoing phenomenon holds a potential for adaptation. The Neurogenic Reserve hypothesis (Kempermann 2008) states that ongoing adult neurogenesis is a special type of brain plasticity that allows for adaptation during activity, that may influence vulnerability to accumulating deleterious events and brain diseases.

While the links between AD and neurogenesis are controversial, connections have been established between neurogenesis, stress, and antidepressant drug action. Of interest, high circulating levels of stress hormones form a substantial risk factor for AD. Maintenance of adult neurogenesis could stabilize synaptic

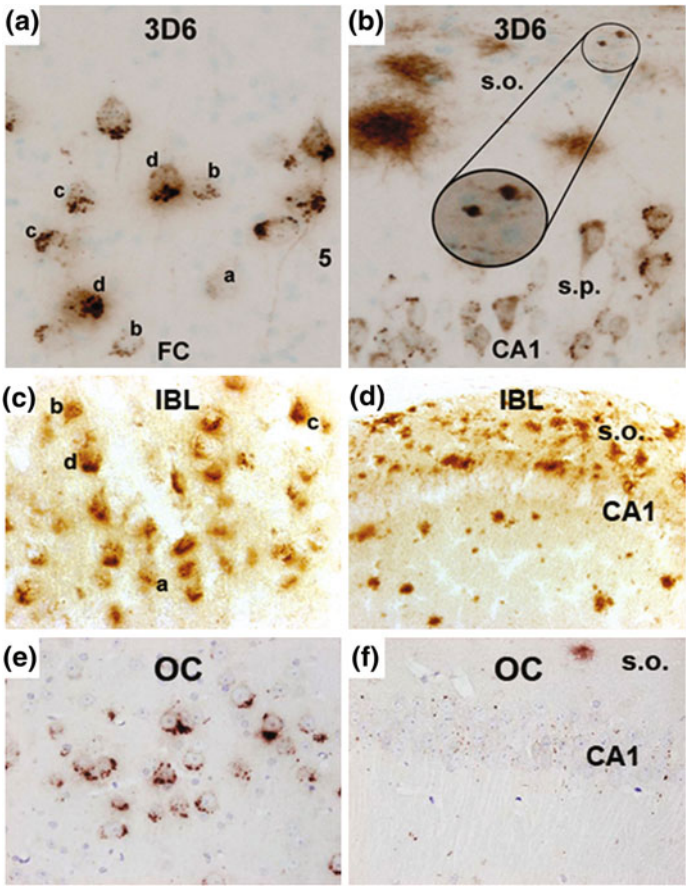

Fig. 9 Comparing cortical and hippocampal Aβ accumulations in aged 3xTg mice during backcrossing with three antibodies (3D6, IBL, and OC) In an effort to identify differences due to backcrossing, this figure compares cortical and hippocampal staining in 3xTg mice. **a** In 20-month-old nonbackcrossed mice, intraneuronal Aβ puncta are condensed in the cortex, increasing density of puncta at the axon terminal is denoted by letters *a-d*. **b** This is also seen to a lesser extent in the CA1, where 3D6 labels neuronal somata; diffuse plaques and axonal processes are also seen in the stratum oriens (*s.o.*) Panels **a**, **b** reproduced from Fig. 2 of (Cai et al. 2012) **c** 20-month-old backcrossed mice, presented in this study, show similar intraneuronal puncta in the cortex with identifiable accumulations at the axon terminals **d** however, no discernable neuronal profiles are seen in the *CA1* of backcrossed 3xTg mice indicating that intraneuronal Aβ is absent in these animals **e** 18-month-old nonbackcrossed mice are positive for intraneuronal Aβ as detected by conformation specific *OC* antibody in the cortex. **f** 3xTg mice on the nonbackcrossed background show low levels of intraneuronal Aβ in the *CA1* when measured with conformation specific *OC* antibody. Panels **e**, **f** adapted from Fig. 4 Wirths et al. 2011

density, alleviating synapse loss, promote structural plasticity, and reduce age-associated changes. Indeed, a loss of haematopoietic stem cells can result in premature aging (Nelson et al. 2012). Currently, however, no evidence has been

collected regarding the relationship between loss of stem cells and specific neurologic dysfunction in humans.

So far, clear age-dependent reductions in neural progenitor cell proliferation have been shown in rodents and old monkeys, and physical activities could reduce this age-dependent decline in precursor cell proliferation. Different models of pathology have shown robust and transient increases in adult cytogenesis that are nonspecific and likely involved in gliogenesis. This nonspecific upregulation or proliferation will at least not result in acute functional recovery. A restricted view of adult neurogenesis implies that for healthy aging to occur it may be perhaps most beneficial, if activity is established in midlife to preserve adult neurogenesis prior to clinical presentation with dementia or AD. As neurons born in aged mammals are just as functional as the ones generated during developmental neurogenesis in young mammals, maintenance of stem cell proliferation, and of the local microenvironment that is responsible for proper migration and connection, is necessary to fully understand the dynamics of the niche during aging and AD.

So far, various animal studies have addressed the relation between the occurrence of Alzheimer pathology and changes in various neurogenesis markers (Verwer et al. 2007; Marlatt and Lucassen 2010). Obviously, these results depend very much on the timing of the onset of pathology, the age of animals studied, and the promotor transgene expressed.

4.5 Presenilin-1 Transgenic Mouse Models

Interest in gamma-secretase and the generation of highly fibrillogenic AB42 has led to the development of presenilin-1 (PS1) transgenic mice expressing mutant PS1. PS1 is part of the gamma-secretase complex but also participates in cellular proliferation, Notch and Wnt signaling mechanisms. PS1 signaling, are responsible for developmental maturation of glia and neurons. In Wnt signaling, PS1 is directly involved with beta-catenin turnover, a mechanism responsible for proliferation of progenitor cells in the developing brain. Normal PS1 facilitates phosphorylation of beta-catenin leading to proteasomal degradation; mutant PS1 cells have increased stability of beta-catenin leading to downstream nuclear signaling events. It is therefore not surprising that neuronal expression of mutant PS1 with a Thy1 promoter increased proliferation in the DG of 4-month-old transgenic mice. Increased cell proliferation did not result in increased neuron survival in the hippocampus of these mice (Wen et al. 2002, 2004; Wang et al. 2004).

Regarding neurogenesis, PS1/PS2 KO mice were evaluated at two ages and found to have increased proliferation and survival. A study of PS1 expressed under the NSE promoter found that proliferation was reduced by both wildtype and mutant PS1. Interestingly, the wild-type PS1 mice had increased survival of immature neurons while the mutants did not though (Wen et al. 2002). A follow-up to this study incorporated groups with environmental enrichment - expression of

the wild-type protein was sufficient to increase survival of immature neurons expressing Tuj1. Enrichment in these mice increased proliferation and survival compared to the nonenriched group. This normal physiology was not preserved in mice expressing mutant PS1; enrichment increased proliferation; however, there were no changes in Tuj1 expression and less surviving BrdU+ cells (Wen et al. 2002, 2004; Wang et al. 2004; Zhang et al. 2007; Kuhn et al. 2007). A more sensitive experiment was produced by crossing mutant PS1 knockin mutants with PS1 knockouts. Mice with one mutant copy of PS1 show impaired learning in a contextual fear conditioning test. This impaired associative learning was positively correlated with impaired neurogenesis. Hence, expression of wild-type PS1 can override the mutant PS1 gene (Wen et al. 2002, 2004; Wang et al. 2004; Zhang et al. 2007; Kuhn et al. 2007).

4.6 Amyloid Precursor Protein Transgenics

Transgenic models of AD further include mice expressing mutant APP; as murine APP does not generate fibrillogenic peptides, typically bigenic mice are generated expressing mutant PS1 and human APP. As reviewed recently, most APP and APP/PS1 mouse models show reductions in cell proliferation, although exceptions exist (Marlatt and Lucassen 2010; Thompson et al. 2008; Kuhn et al. 2007). Limited information is present concerning the subsequent survival of the newborn cells. APP mice had no difference in hippocampal neurogenesis when evaluated by BrdU incorporation at young adult age. At this age the mice do not have amyloid deposits, but when the mice were evaluated at 25 months of age, APP mice exhibited significant increases in the number of BrdU and DCX-positive cells (Ermini et al. 2008) while also in affected human hippocampus and cortex, proliferative and neurogenic-like changes have been seen (Boekhoorn et al. 2006a; Verwer et al. 2007). A separate study utilizing different APP-PS1 mice at 8 months of age showed increased BrdU and NeuN-positive cells compared to controls despite finding that APP-PS1x NestinGFP mice exhibited decreases in nonproliferative Nestin-positive NPCs (Gan et al. 2008). Hence, endogenous neurogenesis appears to be elevated in response to pathology, however, the molecular mechanisms and the functionality of these new neurons are yet to be elucidated.

The relationship between Abeta and neurogenesis has also been combined in interventional studies: environmental enrichment or running was expected to lead to improvements in behavior, via reductions in Abeta plaque load and possibly increases in neurogenesis. As expected, mice provided with environmental enrichment had increases in newborn cell proliferation and survival. These changes corresponded to improved performance in a spatial memory task, but surprisingly, there was no change in plaque load. The results indicate that despite plaque burden, the neurogenic environment is preserved, which allows functional recovery (Wolf et al. 2006; Mu and Gage 2011; Li et al. 2008). Again, structural

pathology appears to dissociate from functional pathology in these models (Mu and Gage 2011; Li et al. 2008; Nelson et al. 2012).

4.7 Tau Transgenic Mice

So far, only a few mouse models of tauopathy have been evaluated. A model utilizing human tau with two mutations describes induction of hyperphosphorylation and the presence of neurofibrillary tangles (NFTs) in 3–6 month-old animals in the hippocampus. Cell bodies of the DG are spared at this young age but neurites in these areas are immunopositive for AT8, indicating aberrant phosphorylation of tau, similar to what is found in AD. Compared to non-Tg mice, transgenic tau mice had 2-fold higher DCX levels and significantly higher expression of TUC-4 in the DG through 6 months (Schindowski et al. 2008). Mice with nonmutant human tau also show signs of proliferation, albeit outside the subgranular zone of the hippocampal dentate gyrus (SGZ) and the subventricular zone (SVZ) (Andorfer et al. 2005). Using a knockout–knock-in approach, it was further shown that expression of 4R tau reduces cell proliferation and increases differentiation and neuronal maturation, confirming an important role for tau in neuronal plasticity and differentiation (Sennvik et al. 2007; Fuster-Matanzo et al. 2012; Llorens-Martin et al. 2012). Also, in young mice carrying the tau-P301L mutation that is associated with frontotemporal lobe dementia, increased long-term potentiation (LTP) in the dentate gyrus was observed, notably parallel to an improved cognitive performance. As neither tau phosphorylation motor deficits nor neurogenesis could account for these changes, this demonstrated that tau plays an important role in hippocampal memory, and that it is not the tau mutations per se, but rather the ensuing hyperphosphorylation that is critical for the development of functional deficits (Boekhoorn et al. 2006b; Fuster-Matanzo et al. 2012; Nelson et al. 2012).

Ultimately, our current study reflects that despite the presence of neuropathology and the lack of behavioral deficits, newborn cell survival can still be stimulated in 3xTg mice by exercise. Notably, this occurs in absence of parallel effects on neuronal differentiation of the newborn cells, neuropathology or hippocampal BDNF levels. Furthermore, backcrossing the 3xTg mouse strain should be avoided as it seems to change its behavioral phenotype, thereby diminishing its predictive value as a model of AD.

Acknowledgments This work was supported in part by the National Institute on Aging (NIA), Intramural Research Program. We thank Dr. Mark Mattson (NIA) for providing the 3xTg mice. MM, TAB, and PJL are supported by the EU (NEURAD consortium). MM and PJL are supported by ISAO. PJL is further supported by the Dutch Brain Foundation, by the International Parkinson Foundation IPF, and by the Netherlands Organization for Scientific Research NWO.

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Part IV
Ageing and Repair

Hippocampal Neurogenesis and Ageing

Sébastien Couillard-Després

Abstract Although significant inconsistencies remain to be clarified, a role for neurogenesis in hippocampal functions, such as cognition, has been suggested by several reports. Yet, investigation in various species of mammals, including humans, revealed that rates of hippocampal neurogenesis are steadily declining with age. The very low levels of hippocampal neurogenesis persisting in the aged brain have been suspected to underlie the cognitive deficits observed in elderly. However, current evidence fails to support the hypothesis that decrease of neurogenesis along normal ageing leads to hippocampal dysfunction. Nevertheless, current studies are suggestive for a distinct role of hippocampal neurogenesis in young versus adult and old brain.

Keywords Cognition · Ageing · Neurogenesis · Doublecortin · Neural stem cell

Contents

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Curr Topics Behav Neurosci (2013) 15: 343–355
DOI: 10.1007/7854_2012_232

Published Online: 14 October 2012

1 Age-Related Neurogenesis Decrease

Although the presence of neurogenesis in the adult central nervous system has been documented in several species of mammals already in the mid-1960s (see for examples early works from Altman and Das 1965a, b, 1966, 1967), true excitement in adult neurogenic processes arose following the confirmation that neurogenesis also takes place in the human adult brain, even beyond the seventh decade (Eriksson et al. 1998). Based on these and other observations, intense effort has been deployed in the recent years to take advantage of the neural stem cells' population sustaining adult neurogenesis to develop regenerative intervention against neuronal loss.

Since the early reports regarding adult neurogenesis, a plethora of studies mainly conducted in rodents revealed that rates of neurogenesis are not steady. Instead, various physiological and molecular regulators have been shown to significant up- and down-regulate neurogenesis by acting on neural stem and progenitor cell proliferation, differentiation and/or maturation (for a review see Ming and Song 2011). Importantly, of all adverse physiological factors acting on neurogenesis, ageing is probably the most potent one. The first detailed report from Kuhn and colleagues revealed that the decrease in hippocampal neurogenesis during ageing is so pronounced that in the dentate gyrus of 21-month-old rats the number of newly added neurons represented only 10 % of the amount detectable in younger rats of 6 months of age (Kuhn et al. 1996). Kuhn and others demonstrated in follow-up studies that the number of newly generated neurons is steadily declining with ageing.

The pronounced age-related decrease in rates of hippocampal neurogenesis observed by Kuhn and colleagues has been meanwhile documented in a broad range of mammalian species, from rodents to human and non-human primates (Amrein et al. 2011; Cameron and McKay 1999a; Couillard-Despres et al. 2009; Gould et al. 1999; Leuner et al. 2007; Lucassen et al. 2010). According to a metaprojection across species, approximately 5 % of all granular cells are proliferating in the mammalian dentate gyrus shortly after birth (Amrein et al. 2011). However, the frequency of proliferating granular cells already declined below 0.5 % within the first 3 months of life according to a logarithmic mode. Moreover, according to the projections of Amrein and colleagues, the pace of decrease of hippocampal neurogenesis is similar across species, irrespective to their lifespan. In consequence, by the time humans transit from adolescence to adult life, proliferating cells in the dentate gyrus would represent < 0.1 % of all granular cells. Hence, the comparatively low level of neurogenesis observed in adult human and primates, as compared to adult rodents, does not imply that postnatal neurogenesis became less relevant along evolution, but simply reflects the longer duration of neurogenesis decrement.

The presence of neurogenesis in the hippocampus constitutes an exquisite form of neuronal plasticity. As compared to other forms, such as synaptic rearrangement, it requires more resources and is more complex in its implementation.

Therefore, it can be assumed that the hippocampus has special plasticity requirement justifying such an investment. Importantly, the bottleneck of neurogenesis' rate lays on a population of neural stem cells, which are by definition unspecialised cells and remain mostly quiescent.

Along ageing, the number of neural stem cells being caught in cell division decreases drastically. Two non-exclusive phenomena could be at the origin of this marked decline. First, the population of neural stem cells might get progressively exhausted due to cell death or differentiation into a specialised cell type. Although, it might be possible to compensate the net output of neurogenesis temporarily by increasing the proliferation of newly generated neuronal precursors or by improving the survival rate of new cells, with the disappearance of neural stem cells, neurogenesis inexorably tappers off. Alternatively, or in addition, the decrease in neurogenesis might be due to unfavourable alterations of the cellular and molecular stem cell niche composition, thereby gradually decreasing the likelihood of stem cells to initiate mitosis with age. In this situation, reinstatement of an adequate environment, for example through a pharmacological intervention, would restore neurogenesis to levels normally associated with youth.

Although the identity of the "real" adult neural stem cell remains elusive, in the dentate gyrus, this function has been ascribed to a group of radial astrocytes (also referred to as type-1 or B cells) spanning the granular layer (Alvarez-Buylla et al. 2002; Kronenberg et al. 2003). Comparison of immunohistological preparations from mouse or rat at different ages reveals that this cell population progressively vanishes (Encinas and Sierra 2012; Encinas et al. 2011). The dispute about the existence of adult neural stem cell maintenance along ageing and the potential to replenish the stem cell population was recently reinflamed by two reports, that came to diametrically opposite conclusions (Bonaguidi et al. 2011; Encinas et al. 2011).

According to a model presented by Encinas and colleagues, adult neural stem cells can be conceived as "disposable stem cells" (Encinas et al. 2011; Encinas and Sierra 2012). This model predicts that once neural stem cells enter proliferation, they undergo successive divisions, generally limited to 2–3 cell cycles, to generate proliferative neuronal precursors. Thereafter, they would terminally differentiate into mature astrocytes. Encinas could not detect evidence for symmetrical division of neural stem cells, i.e. "self-maintenance". In contrast, Bonaguidi and colleagues presented data showing that neural stem cells could, although seldom, divide symmetrically, thereby supporting the tantalising hypothesis that stem cell population might eventually be replenished by a stimulation of neural stem cells promoting symmetrical division (Bonaguidi et al. 2011). A previous report using retroviral infection to follow the fate of Sox2+ cells in the dentate gyrus came to a similar conclusion (Suh et al. 2007). Although Encinas could not exclude that a small population of infrequent proliferating neural stem cells with the capacity of self-renewal might exist, current evidence suggests that under physiological conditions the loss of neural stem cells along ageing is constant and irremediable.

Supplemental decrease of neurogenesis can be observed during neurodegenerative disorders. However, although some age-dependent processes might lead to

neurodegenerative disorder, the latters should not be regarded as accelerated forms of ageing, but discrete physiological disorders. Hence, whereas specific neuronal populations are lost during neurodegenerative disorders, e.g. pyramidal neurons of the CA1 region in the hippocampus of individuals suffering of Alzheimer's disease, no significant granular or pyramidal neuronal loss could be detected along normal ageing in the dentate gyrus and the Ammon's horn (West 1993; West et al. 1994).

Many significant differences in the microenvironment surrounding the neurogenic regions can be documented between the young and the aged brains. Altered levels of neurotransmitter release, the higher corticosteroid concentrations or accumulation of molecules typically associated with inflammation and allergy constitute only few examples of factors that could inhibit neurogenesis and impair hippocampal functions (Cameron and McKay 1999b; Huber et al. 2011; Nichols et al. 2001; Popa-Wagner et al. 2011; Villeda et al. 2011). For example, in cases of stress and ageing, high levels of corticosteroids are thought to inhibit neurogenesis. The fact that adrenalectomized rats, young or old, displayed significant increases of hippocampal neurogenesis supports this assumption (Cameron and McKay 1999b; Spanswick et al. 2011). However, exposition of mice to an enriched environment has been also reported to strongly augment levels of neurogenesis, although levels of corticosterone were threefolds higher than in standard-housed animals (Kempermann et al. 2002). Clearly, regulation of neurogenesis is a complex process summing multiple components. In addition, it must be kept in mind that factors modulating neurogenesis can also act on mature neurons and glia, and thereby profoundly influence hippocampal functions in a neurogenesis-independent fashion.

2 Age-Related Neurogenesis Decrease and Cognition

Should we be concerned by the age-related decrease of neurogenesis within our hippocampi? Whereas the role of neurogenesis in olfaction got somewhat marginal for human survival, processes carried out in the hippocampus, namely cognition and emotion, are the very essence of who we are. At the level of cognitive performances, it is well documented that, on average, human and animal models at advanced age have poorer scores than young adults. However, if neurogenesis levels were directly linked with cognitive performances, the most rapid and pronounced cognitive decline would be observed in first life interval, meaning before puberty, rather than in the elderly population. Several studies attempted to decipher the complex correlation between the age-associated decrease of neurogenesis and the aged-dependent cognitive performance weaknesses. However, no definite evidence has been provided so far that neurogenesis decline in normal ageing causes cognitive deficits.

Despite the fact that ageing is associated with a severe decrease in the rate of hippocampal neurogenesis, in rats, careful analysis in the course of normal ageing ruled out significant neuronal loss (Rapp and Gallagher 1996). In their report,

Rapp and Gallagher used unbiased stereology to quantify the number of neurons within the different hippocampal structures in groups of young and old rats. The latter group was further stratified into groups of good and bad learners according to learning and memory tests (e.g. Morris Water Maze). Yet, despite significant differences in test performances between the three groups analysed, Rapp and Gallagher could not detect any significant difference in the number of neurons as a function of age or cognitive status in the hippocampal regions investigated. Similar histological studies performed in tissues from aged human also came to the conclusion that the number of hippocampal neurons within the dentate gyrus and the Ammon's horn remains stable during normal ageing, although decreases were observed within the hilus and in the subiculum (West 1993; West et al. 1994). These observations substantiate other reports suggesting an equilibrium between cell death and the addition of new neurons within the hippocampus (Biebl et al. 2000; Cameron and McKay 1999b). Hence, the age-associated decrease of hippocampal neurogenesis is most likely compensated by a proportional decrease of cell removal through apoptosis, therefore resulting in a "neuronal *steady state*".

Over more than a decade, several studies aimed to elucidate the function of hippocampal neurogenesis via a targeted depletion of the latter. These studies have been conducted almost exclusively in young animals, which typically display high levels of hippocampal neurogenesis. The first depletion strategies attempted were rather coarse, e.g. cytotoxic agents or X-rays irradiations (see for example Madsen et al. 2003; Shors et al. 2001). The reported learning deficits resulting from these neurogenesis eradications were received with interest, yet with some scepticism because treatments used provoked additional damages and inflammation apart from depleting neurogenesis. Following these pioneer studies, however, numerous works addressed the role of hippocampal neurogenesis using increasingly refined neurogenesis deletion methods and elaborated behavioural testing (for an exhaustive review Deng et al. 2010). Despite all experiments, no clear conclusion on the function of adult hippocampal neurogenesis in cognition can be drawn to date. Although many studies reported correlation between cognitive deficits and total or partial ablation of hippocampal neurogenesis, important discrepancy remains.

Taken that confounding side effects cannot be ruled out for any intervention aiming to deplete neurogenesis, investigation of animal models in which hippocampal neurogenesis is naturally low or absent constitutes an elegant alternative. The cyclin D2 knockout mouse, that lacks adult neurogenesis, is an example of such a useful animal model. However, caution in the interpretation of behavioural tests in this model must be taken since the deletion of cyclin D2 also affects the overall brain development (Jaholkowski et al. 2009; Jedynak et al. 2012; Kowalczyk et al. 2004). In the hippocampus of the cyclin D2 deficient mouse line, only 10 % of the expected number of neuronal precursors (DCX+ cells) can be detected. Noteworthy, the integration of the few neuronal precursors as new mature neurons appears to be completely abolished. Surprisingly, according to a battery of learning-dependent paradigms used by Filipkowski and colleagues, the absence of hippocampal neurogenesis did not overtly affect learning or anxiety

levels in these knockout mice as compared to their wild-type littermates (Jaholkowski et al. 2009; Jedynak et al. 2012). Nevertheless, significant abnormalities in the cyclin D2 deficient mice were detected in some learning-independent behavioural tests, such as nest construction, exploration and motor activities. Taken together, the analysis of the cyclin D2 deficient mouse model strongly suggests that learning is not dependent on the creation of new neuronal circuitries generated in the course of adult neurogenesis. However, it cannot be excluded that normal hippocampal function is supported by the few neuronal precursors present or that more refined behavioural testing would be required to put the cognitive deficits in evidence.

As the cyclin D2 knockout mouse model is also an artificial model, one might rather consider "natural" models of neurogenesis deficiency. For example, some species of shrews and bats are particularly interesting in this respect. Although neurogenesis at the subventricular zone appears to remain life-long in these species, hippocampal neurogenesis can be detected only very early in adulthood and vanishes afterward (Amrein et al. 2007; Bartkowska et al. 2008). Despite the absence of hippocampal neurogenesis, these animals can adapt to an environment in constant mutation, and can memorise precisely the new locations of food supplies, which are spread over large territories in cases of bats. Hence, it appears from these animal models deficient in hippocampal neurogenesis that the generation of new neurons is not required for learning, and in particular spatial learning such as frequently assessed in laboratory setting using variants of the Morris Water Maze task.

Evidence thus indicates that neurogenesis is not essential for learning, yet it might still act as a facilitator. Immature neurons are more readily excitable upon stimuli as compared to their mature counterpart (Couillard-Després et al. 2006; Ge et al. 2007; Schmidt-Hieber et al. 2004). During adult hippocampal neurogenesis, immature neurons are generated in the granular layer of the dentate gyrus, which is the entryway of signals in the hippocampal formation. Hence, due to their strategic position, neuronal precursors could have a significant impact on the sensitivity and response intensity of the dentate gyrus to incoming signals.

Over the last years, several groups developed abstract and biology-based computational models to investigate the roles of the dentate gyrus and neurogenesis in hippocampal functions. One process repeatedly ascribed to the dentate gyrus is the so-called pattern separation (see Aimone and Gage 2011 and Yassa and Stark 2011 for reviews). Pattern separation consists in the generation of contrasted outputs from similar incoming inputs to allow the hippocampus to recognise but distinguish similar, yet different inputs. With ageing, in animal models as well as in human, the efficacy of pattern separation appears to gradually diminish, i.e. greater differences between similar inputs are required to properly recognise the later as "similar" and not "same" (Yassa et al. 2011). The extent of the dentate gyrus' contribution to pattern separation is currently a matter of debates. Nevertheless, it is very unlikely that pattern separation of the constant flow of inputs is performed by the very few new neurons residing in the adult dentate gyrus. Nevertheless, considering their high excitability and their

insensitivity to GABAergic inhibition, the immature neurons could in spite of their poor activation specificity maintain the dentate gyrus and the downward hippocampal circuitry away from an inhibitory stare by "overreacting" to the inputs. Moreover, in contrast to a model system bearing a subpopulation of resident neurons having properties similar to those of young neurons, the solution based on continuous neurogenesis offers the possibility to modulate the number of intervening immature neurons according to the needs.

Along the same line, in a recent in vitro experiment, Stephens and colleague reported that the addition of immature neurons into a mature and stable network led to a rejuvenation of the network activity pattern as a whole (Stephens et al. 2011). According to this study, the activity pattern adopted by the network following addition of immature neurons would facilitate synaptic plasticity and the establishment of LTP, two processes tightly linked to memory formation. Therefore, implantation of neuronal precursors should not, or not only, be regarded as a mean to establish new networks, but also as functional modifier for mature hypoactive or even damaged networks (Stephens et al. 2011). It is worth notice that during ageing, the quantitative decrease of neurons production might be partly compensated by their slower maturation, which *de facto* prolonged the duration of high excitability of the few new cells in the system (Nyffeler et al. 2010; Rao et al. 2005). In 3-month-old mice for example, we reported the presence of NeuN, a marker for mature neurons, in roughly 90 % of granular neurons generated 4 weeks before (Couillard-Després et al. 2006). In contrast, this proportion of NeuN-expressing young neurons was decreased to 8 % when labelling was performed in 20-month-old mice (Kempermann et al. 2002).

3 Neurogenesis Relevance in the Aged Brain

Hence, although neurogenesis rates are continuously decreasing with age, cognitive performances remain rather constant during the adulthood in human and animal models, only showing decline in advanced ages. For example, Cuppini and colleagues investigated groups of rats having 2, 5 and 12 months of age using trace fear conditioning, which assesses hippocampal-dependent learning (Cuppini et al. 2006). As expected, levels of hippocampal neurogenesis was significantly reduced in the two older groups of rats, as compared to the 2-month-old rats, with reduction roughly representing 50 and 95 %, respectively. Notwithstanding the massive depletion of hippocampal neurogenesis in older rats, no significant differences in their test performance could be detected between the three groups (Cuppini et al. 2006). Intriguingly, a decrease of approximately 50 % of hippocampal neurogenesis in young adult rats via the application of a cytostatic agent, methylazoxymethanol (MAM), resulted in a significantly poorer test performance (Shors et al. 2001). How can this behavioural discrepancy be explained? Are poorer performances resulting from a broad cytotoxic effect upon MAM application, or do young and old animals differ in their requirement of neurogenesis for hippocampal functions?

As the functions of adult neurogenesis are not yet deciphered, speculation on the consequence of neurogenesis modulation such as during ageing is particularly precarious. Evidence accumulated so far from the various investigations on hippocampal neurogenesis argues against the simple correlation "the more, the better". Moreover, the fact that sites of integration for newly generated neurons are also sites of apoptosis, suggests that mere addition of supernumerary neurons does not constitute the aim of adult neurogenesis. Instead, the possibility to integrate new neurons could enable the continuous selection for neurons that are functionally better suited for the current spectrum of tasks to be accomplished and can be envisaged as the capacity of original circuitries to become gradually optimised.

In order to better pinpoint the impact of neurogenesis on hippocampal function, it is advantageous to minimise the number of intervening variables, such as age or treatment and rely only on the interindividual variation of neurogenesis rates. Using such a strategy, Bizon and colleagues came in 2004 to the astonishing observation that the levels of neurogenesis in the population of older rats were inversely proportional to their learning index, i.e. good learners had less hippocampal neurogenesis than bad learners (Bizon et al. 2004). In a more recent report, this finding was readdressed by Nyffeler and colleagues who looked at ongoing neurogenesis by quantifying the population of neuronal precursors expressing DCX in rats of three different age groups (3, 6 and 24 months of age) (Nyffeler et al. 2010). At first sight, comparing all groups at once, a correlation appeared between learning performance and higher neurogenesis levels. However, following partition of rats within their respective age groups a completely different picture arose. Hence, replotting the data generated by Nyffeler, Lazic observed that direct correlation between learning and neurogenesis could only be detected in the 3-month-old rat group, whereas in the 6- and 24-month-old groups, an inverse correlation was observed, consistent with the report of Bizon and colleagues (Lazic 2010).

Once more, do requirements in neurogenesis for adequate hippocampal function differ in young and old individuals? Although the cellular composition of the hippocampus appears to remain relatively stable from the time point at which development is completed up to an advanced age, the young hippocampus can still be considered as immature system since it "lacks experience". A similarity could be perceived with the immune system. In addition to the cellular development of the hippocampus, further maturation will result from the constant flow of inputs coming from the environment. The hippocampal circuitry will "learn", will get functionally optimised to the needs and will progressively accumulate experience. In this respect, neurogenesis would enable the possibility to select, out of the newly generated population, neurons better suited for hippocampal function. This process might be critical in young immature individuals, but dispensable in the older and already optimised hippocampus.

Similar to the neuronal content, the overall thickness of the molecular layer remains constant over most of the life span, but undergoes qualitative changes. Hence, in aged rats, a significant reduction of the medial molecular layer (incoming projections from the medial entorhinal cortex) to the benefit of the inner molecular layer (commissural/associational inputs arising from hilar neurons) was

reported (Rapp et al. 1999). These track volumetric changes along ageing reflect the synaptic reorganization and permanent rewiring of the hippocampus. It is tempting to conclude that along ageing, processing of incoming inputs will increasingly involve association to previously acquired memory. Investigation of the dynamic of such hippocampal circuitry rewiring in species showing very low or no adult neurogenesis might reveal if this constitute a common strategy to overcome the decreased number of immature neurons production.

Similar functional rewiring was suggested by a recent fMRI investigation comparing two groups composed of men with 20 or 60 years of age (Burgmans et al. 2010). Although these two groups were shown to have equivalent cognitive performance according to the tests employed, differences were observed in cortical activation patterns during memory processing. Hence, the aged group revealed in fMRI a weaker activation of the medial temporal areas, as compared to the 20-year-old candidates, whereas the ventral and prefrontal cortex, as well as in some additional frontal and parietal regions presented a stronger activation (Burgmans et al. 2010; Grady 2008). One could speculate that individuals of 60 years of age relied for the execution of cognitive challenges more heavily on association with previously acquired experiences.

It is noteworthy that in numerous studies stronger challenge of the cognitive resources in youth and adulthood appears to lower the risk of cognitive decline during ageing, and reciprocally lower educational attainment is consistently associated with higher risks of dementia (e.g. Tervo et al. 2004). One interpretation could be that intense cognitive stimulation during the phase of high neurogenesis levels would permit a thorough optimisation of hippocampal processing and allow to mask longer functional deficits associated with pathological processes. This postulation would be in line with the cognitive and the neurogenic reserve hypotheses. The cognitive reserve hypothesis states that optimised neuronal circuitries have greater functional capacities and are less susceptible to disruption *per se* and/or can better compensate for dysfunctional elements (Kempermann 2008; Stern 2006). On the other side, the neurogenic reserve hypothesis implies that the generation of new neurons constitutes a cellular plasticity to incessantly optimise the hippocampal circuitry according to the demand. As younger individuals are relatively "inexperienced" as compared to elders, there is a stronger need for optimisation of input processing and therefore more need for neurogenesis. Once optimised, the network remains more efficient over long periods of time for the processing of similar inputs. Following a context change, e.g. enriched environment, neurogenesis can be stimulated, even in the aged hippocampus (Kempermann et al. 1998). Therewith, increased neurogenesis provides new resources for greater cellular plasticity as required in the new situation. However, as the neurogenesis levels are constantly decreasing along ageing, the reserve available for optimisation inexorably thins out as well.

In any case, interpretation of such a correlation between risk of dementia and educational attainment must be taken with great care. Hence, persons susceptible to develop dementia might be already biased against intensive cognitive challenges long before the appearance of the first symptomatic manifestation.

Since the first reports on age-related neurogenesis decrease, many strategies have been envisaged to counteract neurogenesis decline with pro-neurogenic approaches. In light of actual evidence, however, the pertinence and potential benefit of such treatment must be questioned. The common finding of a decrease in neurogenesis in ageing mammals supports the idea that it does not represent a major evolutionary disadvantage (Amrein et al. 2011). Moreover, would an undue increase of neurogenesis in the aged brain be detrimental to a system that has been optimised over a long period of time? Usefulness of targeted neurogenesis stimulation might be limited to pathological situations in which neuronal loss and dysfunction are involved and require mending. As for normal ageing, it might simply be the best to make good use of available resources today in order to optimise our brain for tomorrow.

Carpe diem

Acknowledgments The author is grateful to Prof. L. Aigner (Paracelsus Medical University, Salzburg) and Prof. B. Iglseder (Christian Doppler Clinic, Salzburg) for their critical and stimulating comments. This work has been made possible through the support from the State Government of Salzburg (Austria), the German Federal Ministry of Education and Research (BMBF grant 01GN0978) and the foundation Propter Homines (Liechtenstein). The research leading to these publication has received funding from the European Union's Seventh Framework Programme (FP7/2007-2013) under grant agreement n° HEALTH-F2-2011-278850 (INMiND) and n° HEALTH-F2-2011-279288 (IDEA).

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Repair of the CNS Using Endogenous and Transplanted Neural Stem Cells

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Abstract Restoration of the damaged central nervous system is a vast challenge. However, there is a great need for research into this topic, due to the prevalence of central nervous system disorders and the devastating impact they have on people's lives. A number of strategies are being examined to achieve this goal, including cell replacement therapy, enhancement of endogenous plasticity and the recruitment of endogenous neurogenesis. The current chapter reviews this topic within the context of Parkinson's disease, Huntington's disease and stroke. For each disease exogenous cell therapies are discussed including primary (foetal) cell transplants, neural stem cells, induced pluripotent stem cells and marrow stromal cells. This chapter highlights the different mechanistic approaches of cell replacement therapy versus cells that deliver neurotropic factors, or enhance the endogenous production of these factors. Evidence of exogenously transplanted cells functionally integrating into the host brain, replacing cells, and having a behavioural benefit are discussed, along with the ability of some cell sources to stimulate endogenous neuroprotective and restorative events. Alongside exogenous cell therapy, the role of endogenous neurogenesis in each of the three diseases is outlined and methods to enhance this phenomenon are discussed.

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Curr Topics Behav Neurosci (2013) 15: 357–398
DOI: 10.1007/7854_2012_223

Published Online: 21 August 2012

Keywords Stroke · Huntington's disease · Parkinson's disease · Neural transplantation · Stem cell · Neurogenesis · Cell therapy

Abbreviations

6-OHDA	6-hydroxydopamine
BBB	Blood–brain barrier
BDNF	Brain-derived neurotrophic factor
bNGF	Nerve growth factor beta
DA	Dopamine
DAergic	Dopaminergic
ESCs	Embryonic stem cells
FGF	Fibroblast growth factor
GDNF	Glial cell line-derived growth factor
GE	Ganglionic eminence
HD	Huntington's disease
iNs	Induced neural stem cells
iPSCs	Induced pluripotent stem cells
L-DOPA	L-dihydroxyphenylalanine
MPTP	1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine
MSCs	Mesenchymal stem cells
NSCs	Neural stem cells
PD	Parkinson's disease
SGZ	Subgranular zone
SN	Substantia nigra
SVZ	Subventricular zone
TH	Tyrosine hydroxylase
VEGF	Vascular endothelial growth factor
VM	Ventral mesencephalon

Contents

1 Introduction

Following W. Thompson's (1890) first report of adult neural tissue transplantation in the brain, advances in neural transplantation have accelerated. Approximately 20 years later, Elizabeth Dunn found much better survival with embryonic tissue transplantation into the neonatal rat brain (Dunn 1917), and since then the field has grown exponentially, with multiple cell sources, neuroanatomical systems, and applications in a diverse range of neurological diseases examined. These cell sources include primary foetal tissue, immortalised cell lines, embryonic stem cells (ESCs, derived from the blastocyst stage during development), neural stem cells (NSCs, either derived from embryonic, foetal or adult tissue), induced pluripotent stem cells (iPSCs, e.g. derived from adult fibroblasts) or stem cells derived from other somatic tissues including bone marrow, adipose tissue, dental pulp, umbilical cord blood, placenta or menstrual blood. This chapter will consider recent advances in the development and application of exogenous and endogenous cell therapies, with a specific focus on Parkinson's disease (PD), Huntington's disease (HD) and ischaemic stroke.

1.1 Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder characterised by its cardinal motor symptoms, i.e., akinesia and bradykinesia, which stem from a progressive loss of dopaminergic (DAergic) neurons in the substantia nigra (SN) and the associated loss of dopamine (DA) in the striatum (see: Jellinger 2012). Patients with the disorder also suffer from a variety of non-motor symptoms, such as autonomic dysfunction, olfactory deficits, cognitive impairment and depression which cannot be explained by exclusive nigral pathology (reviewed in Lindgren and Dunnett 2012). Intensive research during the last 40 years has resulted in the development of effective DA replacement therapies, including the precursor L-dihydroxyphenylalanine (L-DOPA) and, more recently, clinical trials using cell transplantation of foetal ventral mesencephalon (VM) tissue (the region of the developing foetal midbrain from which the nigral DAergic neurons originate). In contrast, stimulation of endogenous neural stem cells or transplantation of DAergic neuroblasts derived from other sources than human foetal VM (e.g. stem cells), are still in a relatively early phase of development (Kim 2011).

1.1.1 Primary Foetal Cells

By the end of the 1970s, Anders Björklund's lab at the University of Lund, Sweden, had developed novel strategies to transplant foetal (primary) donor tissue into the brain. The cells from this tissue are not stem cells, as they have already

undergone differentiation to specify their neuronal phenotype, but they still require further growth, maturation and the development of neurite outgrowth. Björklund and colleagues realised the potential of utilising this cell source for transplantation and embarked upon an investigation of the functional capacity of these grafts. The idea of 'brain repair' was postulated, and the hope of offering cell replacement therapies in PD was born. In 1979, Björklund and Stenevi, as well as another American-Swedish research collaboration (Perlow et al. 1979), reported independently that DA-rich grafts were capable of alleviating motor impairments in a rat model of PD. These two pioneering studies paved the road for the field of neurotransplantation, and by the following decade numerous publications were available that evaluated the impact of neural grafts on motor and non-motor behaviours in animal models of various neurological conditions (for summary see Dunnett 2010).

For PD, the 'gold standard' primary cell transplants are harvested from the VM of a developing foetus. The tissue is prepared, either as blocks of tissue or a cell suspension. Using stereotaxic techniques, this preparation is then ectopically transplanted into the DA-depleted striatum, rather than the SN, which is the site of cell degeneration. Ectopic transplants are utilised due to considerable evidence suggesting that grafted DAergic neurons are unable to grow sufficient projections from the SN to re-innervate the distant striatum. However, some recent work and alternative methods of transplantation have been promising, insofar as considerable enhancement of the outgrowth from grafts placed in the SN has been demonstrated (Strömberg et al. 2001; Thompson et al. 2009). These transplantation experiments, and the majority of preclinical studies, have primarily been conducted using the rat 6-hydroxydopamine (6-OHDA) lesion model of PD (Ungerstedt 1968), which simulates late stage PD with an almost full depletion of DA innervation to the striatum in one hemisphere. Behavioural analysis of this model reveals lesion-induced impairments and allows for the assessment of the functional efficacy of cell replacement therapies. One such test, drug-induced rotation (Ungerstedt and Arbuthnott 1970), consists of systemically injecting the presynaptic stimulant amphetamine and evaluating the rotational bias caused by the unilateral DA depletion. VM-grafted animals show full recovery after transplantation in this test, i.e. grafted rats have no rotational bias anymore or even overcompensate by rotating to the other side (Björklund et al. 1980). It has also been demonstrated that the surgical removal of the graft returns rotational bias to levels of pre-transplantation, indicating that graft survival and function are necessary for a reduction of rotational bias (Björklund et al. 1980; Dunnett et al. 1983). Although this test provides a useful assessment of graft functionality, it essentially measures the basic capability of the grafted cells to release DA, rather than restoring a natural DA-dependent behaviour. Other simple motor tests based on more natural behaviours also responded positively to DA grafts in a rat PD paradigm, including sensori-motor tests such as the adjusting step and vibrissae-evoked hand-placing test, as well as those that measure motor behaviour, such as rotarod, akinesia, cylinder, or catalepsy tests (see Dunnett and Björklund 2010 for more detailed review). These tests are also commonly applied to the models of HD and stroke. An important concept learnt from such grafting studies

is that the striatal circuitry—similar to the cortical motor map—is heterogeneously and topographically organised (Metz et al. 2004; Klein et al. 2007; Jungnickel et al. 2011), requiring distinct graft placement into brain areas that are critical for performing different behaviours (Dunnett et al. 1983).

Restoration of skilled motor function, particularly skilled reaching deficits, has been more challenging than achieving improvements in basic motor behaviours. Early studies using skilled reaching tests failed to show any benefit, despite good graft integration and survival (Dunnett et al. 1987; Montoya et al. 1990; Abrous et al. 1993). In this case, these failures were independent of the topographical placement of the graft into the striatum, suggesting that a fully functional nigro-striatal pathway and somatosensory feedback was needed to restore skilled hand use (Dunnett et al. 1987; Wictorin 1992; Wilby et al. 1999). Poor survival, incomplete electrophysiological and morphological maturation (though sufficient to improve other less challenging and simple behavioural tests, such as drug-induced rotation), and the ectopic placement of DA neurons have been suggested to be responsible for weak results in skilled reaching. There was hope that bridge grafts, guiding axonal outgrowth from transplanted cells towards their target area, would achieve the big break-through, but they also failed to show any major improvements in more complex behaviours (Dunnett et al. 1989; Wictorin 1992; Mendez et al. 1996; Wilby et al. 1999; Winkler et al. 2000). Other groups, however, were able to identify mild to substantial improvement by optimising the experimental protocols, for example, by implementing a microtransplantation technique using a glass capillary to minimise scar formation in the host and modifying test parameters by adjusting behavioural analyses to pick up subtle graft-related changes (Nikkhah et al. 1998; Garcia et al. 2011; Klein et al. 2012).

In line with these behavioural changes, it has been demonstrated that the physiological correlate to the observed behavioural restorative capacity of the grafts was the ability to restore DA levels after transplantation into 6-OHDA lesion rats (Björklund et al. 1980; Freed et al. 1980; Schmidt et al. 1982). Electrophysiological firing profiles of grafted neurons were demonstrated (Wuerthele et al. 1981), and the grafted neurons could form synaptic connections with host neurons (Freund et al. 1985). Moreover, the grafts restored in vivo release of DA into the host striatum at physiological levels and under local regulation (Zetterström et al. 1986; Strecker et al. 1987; Cragg et al. 2000).

Although primary VM-derived transplants have shown the capability to improve motor function in both PD patients and rodent models of the disorder, the effects on cognitive symptoms are less clear. Only a few clinical studies have looked into more cognitive-related functions associated with transplants in PD patients, and whereas one study indicated that transplants may have a beneficial effect (Sass et al. 1995), another reported no change in cognitive performance after grafting (Trott et al. 2003). Preclinical data on the effect of DAergic grafts on cognition are also very limited. Taylor and co-workers demonstrated an improvement in object recognition after nigral transplants into the striatum of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated monkeys (Taylor et al. 1990), and another study has been investigating the effect of grafting on

emotional measures in DA-depleted rats (Jungnickel et al. 2011). DAergic transplants have also been shown to functionally restore the impairment on a lateralised choice reaction time task seen after a 6-OHDA lesion in rats (Dowd and Dunnett 2004). However, neural transplantation for cognitive impairment is still in its infancy, and further studies, both preclinical and clinical, are necessary in order to elucidate the full effect of transplants on cognitive functions.

Given the extensive evidence of improvements in basic sensory and motor behaviour, as well as more complex processes such as skilled reaching or cognitive tasks, it is clear that the grafting of primary foetal cells has been the most successful type of brain repair so far achieved. However, there is also a need to develop new cell sources for this therapy due to the ethical, logistical and quality control concerns surrounding the use of foetal tissue. For example, five to six aborted embryos are required per hemisphere of a PD patient. Logistically, this does not make primary tissue a viable wide-scale treatment, and some people express ethical concerns over the use of embryonic tissue. Therefore, much work has more recently been devoted to developing DAergic neurons in vitro from a number of novel cell sources.

1.1.2 Neural and Embryonic Stem Cells

Stem cells have been developed as an alternative cell source for brain repair in PD, to remove some of the issues associated with the use of primary foetal tissue for cell replacement therapy. Stem cells are attractive for neurorestorative approaches for various reasons: they theoretically have unlimited proliferative ability, and established propagation and differentiation protocols in vitro make them a good candidate to harvest enough cells prior to transplantation and differentiate them into the desired cell type. Once fully differentiated, stem cells have a small or no risk of tumour formation. However, in practice the maturation of cells in vitro is not the same as in vivo, making definitive knowledge of the stage of cells difficult even within a fairly mature population. There is still ethical dispute attached to the use of ESCs, as they are harvested from the blastocyst stage of the fertilised egg. There are also issues with the efficacy of differentiation into the desired cell type, specifically into the DAergic nigral phenotype, which is limited. Evidence of functional restoration in in vivo models has been sparse, thus far (Bjorklund et al. 2002; Kim et al. 2002), in comparison to the transplantation of primary cells (see above). Another obstacle for using in vitro generated cells for transplantation is that long-term propagation drives stem cells towards a glial or non-DAergic cell fate which could potentially compromise a clinical use of ESC and NSC lines (Jain et al. 2003).

NSCs are traditionally considered to be self-renewing multipotent cells, which are driven down a neural linage and can differentiate only into the phenotype of cells which are present in this lineage, i.e. neurons and glia; however, it has been found that, if the right environmental factors are provided, they can give rise to many non-neural cell types as well (Price and Williams 2001). The term ‘multipotent’ can be used to cover a number of different cells from various sources. For

example, adult, foetal or ESCs that have been cultured can be multipotent, self-renewing NSCs. NSCs from rat foetal VM have been identified and cultured in vitro since the early 1990s (Reynolds and Weiss 1992), and have been of particular interest for neurotransplantation in PD. Recent publications report an increased yield of DAergic cells following distinct differentiation protocols by using mitogens such as sonic hedgehog (SHH), fibroblast growth factor (FGF) two or eight (For a review see: Smidt and Burbach 2007). The transplantation of rat-derived, as well as human-derived, NSCs has resulted in behavioural improvement of motor deficits (e.g. less rotational bias after transplantation), yet the effects with human cells are limited and less obvious compared to rodent-derived cells (Svendsen et al. 1997; Studer et al. 1998; Sánchez-Pernaute et al. 2001; Yan et al. 2001; O'Keeffe et al. 2008). Non-mesencephalic NSCs can also serve as a source for DAergic cells; it is, however, more challenging to achieve a large yield of cells with a DAergic phenotype compared to mesencephalon-derived NSCs, which might be due to epigenetic differences between the origins of NSCs (Rössler et al. 2010). Pre-sorting of DA cells ex vivo, using fluorescence-activated cell sorting (FACS), before transplantation is another option to increase the DA cell survival within the grafts (Donaldson et al. 2005; Hedlund et al. 2008).

Pluripotent ESCs have been harvested from murine blastocysts and were described first more than three decades ago (Evans and Kaufman 1981; Martin 1981). Cultivation of primate and human ESCs was reported 14 and 17 years later, respectively (Thomson et al. 1995, 1998). Although ESCs can be successfully propagated and differentiated into DAergic cells to a higher degree than NSCs, (Lee et al. 2000; Kim et al. 2002; Cho et al. 2008), the survival of ESC-derived DAergic cells within the graft remained relatively low. However, grafts displayed a good integration into the host tissue in general (Kim et al. 2002; Ben-Hur et al. 2004; Brederlau et al. 2006). Despite the low number of cells with DAergic phenotype surviving the transplantation process, functional benefits post-transplantation were observed in a rat model of PD (Kim et al. 2002; Yang et al. 2008).

A major disadvantage of ESCs though, is that it is impossible to fully exclude non-differentiated proliferating cells during cell preparation for transplantation. ESC suspensions ready for transplantation surgery have a high risk of tumour formation due to the possibility of even a few non-differentiated proliferating (stem) cells contaminating an otherwise fully differentiated preparation (Brederlau et al. 2006; Kim et al. 2009). Extended differentiation prior to transplantation further reduces the already low survival of DA cells within the grafts (Jönsson et al. 2009), but—consequently—also reduces the risk of tumour growth.

It is important to note that primary cells, NSCs, and ESCs are not autologous to a potential recipient and can activate the host immune system. Although, the brain exhibits a degree of immunological privilege, such that 'allogeneic' neural grafts within species can survive without immune suppression, when transplanted between species 'xenograft' cells will always be rejected after transplantation, even into the brain. Hence, for evaluating human neural tissue transplants in animal models of disease, immunosuppressive drug treatment is necessary, which, in turn, causes major aversive side effects in the rodent host or the patient. To circumvent these issues, efforts

have been made to develop alternative immune protection strategies (Kelly et al. 2009), to use autologous transplantation of iPSCs and induced neural stem cells (iN cells) that can be harvested from, for example, the host's own fibroblasts, or to adopt other endogenous approaches to brain repair, e.g., by stimulating neurogenesis.

1.1.3 Induced Pluripotent Stem Cells

Autologous transplantation is an interesting approach to brain repair. Logistical, ethical and immunological issues with primary and stem cells are a big hurdle in developing a therapy that can be commonly used. Hence, the advantages in this approach are obvious: patients could donate their own cells (e.g. skin fibroblasts), and thereby avoid any immunogenic reaction to a graft, and the ethical issues associated with the use of autologous cells are also less controversial.

iPSCs were described first in murine cells by Takahashi and Yamanaka (2006). The concept of driving adult non-neuronal cells into a neural phenotype was revolutionary and a crucial step forward in stem cell biology and brain repair. The initial publication used a viral transfection technique and overexpression of certain growth factors to reprogram fibroblasts into ESC-like cells (Takahashi et al. 2007a). This method was successfully established in human-derived cells as well (Takahashi et al. 2007b), and protocols have subsequently been refined using non-viral transfecting systems and excluding potentially carcinogenic factors (Nakagawa et al. 2008; Stadtfeld et al. 2008; Soldner et al. 2009; Woltjen et al. 2009; Zhou et al. 2009; Jia et al. 2010). Interestingly, iPSCs derived from patients suffering from a genetic form of PD could be propagated in vitro as well and could serve as a future in vitro preclinical model for testing e.g. neuroprotective agents in the cell culture dish rather than using animals for research (Park et al. 2008; Soldner et al. 2009). However, this is a drawback of using a PD patient's own cells for transplantation, where the therapeutic cells for transplantation may express the detrimental mutation which caused the disease.

Successfully reprogrammed cells display growth patterns, gene expression and morphology similar to ESCs, including the risk of teratoma formation. Murine and human iPSCs both have been differentiated into DA neurons (Cai et al. 2010; Swistowski et al. 2010) and grafted into hemiparkinsonian rats (Wernig et al. 2008; Cai et al. 2010; Hargus et al. 2010). The cells showed substantial survival in vivo, produced some remarkable behavioural recovery, but also, in some cases and as expected, they formed teratomas (Wernig et al. 2008; Dyson and Barker 2011). It is believed that those teratomas derived from non-differentiated iPSCs in the injected cell suspension. As with the ESCs, FACS technology might aid in the exclusion of immature cells to avoid/reduce tumour formation in the future.

Excitingly, it has recently been shown that the direct conversion of fibroblasts into neurons (so-called iN cells) can be induced by reprogramming cells into the desired cell type, i.e. a neural phenotype, without the embryonic stem

cell-like stage. Hence, this method avoids the risks of tumorogenesis, which is in contrast to the high risks of tumour formation after transplantation of iPSCs or ESCs. Neuronal cell characteristics of those iN cells have been recorded; neurorestorative capacities in vivo, however, need to be further investigated (Vierbuchen et al. 2010; Pfisterer et al. 2011; for review see Vierbuchen and Wernig 2011).

1.1.4 Endogenous Neurogenesis

Neurogenesis, defined as the process of generating functional neurons from precursors, was long believed to occur only during the embryonic and perinatal stages (reviewed in Ming and Song 2005). However, although Altman and colleagues provided evidence of adult neurogenesis as early as 1965 (Altman and Das 1965), it was not until the 1990s that a general acceptance of functional neurogenesis in the central nervous system (CNS) was achieved (Reynolds and Weiss 1992). Active adult neurogenesis occurs mainly in the subgranular zone (SGZ) in the dentate gyrus of the hippocampus and the subventricular zone (SVZ) of the lateral ventricle. Neurons born in the SGZ differentiate into granular neurons and integrate into the local network in the dentate gyrus (Kuhn et al. 1996), whereas SVZ neurons migrate through the rostral migratory stream and become granule neurons of the olfactory bulb (Lois and Alvarez-Buylla 1993, reviewed in Gage 2000). Although extensive research has resulted in greater understanding of the processes involved in adult neurogenesis, its functional importance is still under debate. Analyses at cellular, circuitry and behavioural levels have resulted in accumulated evidence supporting the contribution of neurogenesis to olfactory function as well as learning and memory (reviewed in Aimone et al. 2011; Lazarini and Lledo 2011).

The SVZ is located adjacent to the striatum and is highly innervated by DAergic terminals from the SN (Höglinger et al. 2004). A reduction in proliferation in the SVZ, as a result of the DA depletion, has been demonstrated in both rat and mouse models of PD (Höglinger et al. 2004; Baker et al. 2004; O'Keeffe et al. 2009) as well as in PD patients (Höglinger et al. 2004; O'Keeffe et al. 2009). Interestingly, the reduction of proliferation is correlated to the extent of the DAergic lesion in the neighbouring striatum (Baker et al. 2004) and gradually returns back to baseline levels during the time of DA re-innervation after MPTP injections (Höglinger et al. 2004). During embryonic development, the neurotransmitter DA and its receptors (D1-like receptors: D1 and D5; D2-like receptors; D2, D3 and D4) are present in the highly proliferative germinal zones of the brain (Voorn et al. 1988; Ohtani et al. 2003). The subsequent activation of specific intracellular pathways has been demonstrated to play dual roles in embryonic neurogenesis. More specifically, D2-like receptors are involved in promoting the proliferative effect of DA, whereas the D1-like receptors have an antagonising effect on proliferation at the level of cell cycle entry (Diaz et al. 1997; Ohtani et al. 2003). In the adult brain, the D2-like receptors are found on the rapidly dividing

precursor cells of the SVZ, whereas the D1-like receptors are expressed to a much lesser extent (Höglinger et al. 2004; Coronas et al. 2004; Kippin et al. 2005). Therefore, it is not surprising that drugs acting on D2-like receptors such as L-DOPA (O'Keeffe et al. 2009) or agonists such as ropinirole (Höglinger et al. 2004), and pramipexole (Winner et al. 2009) have the capacity to restore the precursor proliferation in the SVZ back to baseline levels.

Altered proliferation has also been observed in several α-synuclein transgenic mouse models of PD, overexpressing both the wildtype and mutated form of human α-synuclein under various promoters (Winner et al. 2004; Crews et al. 2008; Nuber et al. 2008; Marxreiter et al. 2009). The reduction in neurogenesis was evident in both the dentate gyrus and in the SVZ/olfactory bulb systems, and coupled to α-synuclein overexpression, since the reduction of neurogenesis could be modulated by transiently switching off α-synuclein expression using conditional mouse models (Nuber et al. 2008; Marxreiter et al. 2009).

Residing cells within the striatum and the SN also hold a proliferative potential, and increased proliferation in these regions has been demonstrated after both MPTP and 6-OHDA lesions. The majority of these new-born cells differentiate into a glial phenotype particularly around the lesion site, whereas some of the cells remain as uncommitted progenitors (Kay and Blum 2000; Mao et al. 2001; Steiner et al. 2006; Kitamura et al. 2010). Interestingly, tyrosine hydroxylase (TH)-positive cells have been found in the adult striatum after DA depletion in both rodent and primate models of PD (Meredith et al. 1999; Petroske et al. 2001; Palfi et al. 2002), as well as in PD patients (Porritt et al. 2000). However, these cells do not stain positively for 5-bromo-2̈-deoxyuridine (BrdU; a marker of new-born cells), which suggests that they represent striatal cells undergoing a phenotypic switch as a result of the DAergic denervation rather than being born in situ. Intriguingly, these TH-positive cells are responsive to L-DOPA and increase both in number and intensity after chronic treatment in 6-OHDA-lesioned mice (Francardo et al. 2011; Smith et al. 2012). The mechanism underlying the phenotypic switch as well as their functional importance remains to be demonstrated.

The functional implication of the reduction in neurogenesis seen after DA depletion remains speculative. PD has long been considered to be a pure motor disorder, but it has recently become evident that PD patients also experience a variety of intrinsic non-motor symptoms. Some of them, such as olfactory deficits and depression, may even precede the onset of motor symptoms by several years (reviewed in Lindgren and Dunnett 2012; Doty 2012). Interestingly, the pathological changes underlying these symptoms may be located in the two neurogenic regions discussed above.

There is increasing evidence that altered adult neurogenesis in the SVZ/olfactory bulb system modifies olfactory processing in many ways, mainly by effecting cognitive olfactory functions rather than sensory processing per se (reviewed in Lazarini and Lledo 2011). The olfactory deficit in PD also is dependent on central olfactory functions (Silveira-Moriyama et al. 2009), and an increased number of DAergic cells has been demonstrated in the olfactory bulb of PD patients (Huisman et al. 2004; Mundinano et al. 2011) as well as in rodent

models of the disorder (Yamada et al. 2004; Winner et al. 2006). These findings suggest that increased DAergic activity in the olfactory bulb, rather than loss of DA, may be contributing to the olfactory deficits seen in PD.

A "neurogenic" theory also exists for the link between decreased hippocampal neurogenesis and the vulnerability for depression as seen in PD patients, which is based on the fact that increased levels of stress hormones reduces the levels of adult neurogenesis in the hippocampus (reviewed in Samuels and Hen 2011). This theory was strengthened by the finding that the newborn neurons in the dentate gyrus mediated some of the beneficial effects seen after anti-depressant treatment (Santarelli et al. 2003; Sahay and Hen 2007; David et al. 2009). Altered hippocampal cell proliferation has been demonstrated in a few rodent models of PD (Crews et al. 2008; Kohl et al. 2012; Nuber et al. 2008; Park and Enikolopov 2010; Winner et al. 2004), but whether hippocampal neurogenesis is affected in PD patients or not remains unknown.

The existence of endogenous neurogenesis paved the road for new therapeutic approaches as an alternative type of cell-based neurorestorative approach for the treatment of PD. As for the majority of existing therapies, symptomatic relief of (motor) deficits can be achieved by increasing the levels of DA in the striatum. It would be an immense step forward in (non-invasive) brain repair, if neuronal precursors from the SVZ could be recruited or if the resident cells within in the structure could be stimulated to differentiate into DA-releasing neurons. Recruitment of neuronal precursors from the SVZ to the striatum has proven possible in various animal models of PD using the administration of growth factors, such as the glial cell line-derived growth factor (GDNF; Palfi et al. 2002) and the transforming growth factor-α (Fallon et al. 2000). It should be noted, however, that the functional implication of neural precursor recruitment from the SVZ to the striatum has yet to be demonstrated.

Another endogenous restorative approach would be to alter the local microenvironment in the SN into one that would favour a neuronal differentiation of dividing cells. Several studies have demonstrated pronounced cellular plasticity in the SN after DAergic denervation with increased proliferation. It was first suggested that a subset of proliferating cells in the SN after a 6-OHDA lesion could differentiate into DAergic neurons (Zhao et al. 2003), however, it has more recently been established that the vast majority of the proliferating cells in this region differentiate into a glial phenotype, particularly around the lesion site, rather than into neurons (Kitamura et al. 2010; Kay and Blum 2000; Mao et al. 2001; Frielingsdorf et al. 2004; Steiner et al. 2006). If these glial cells are expanded ex vivo or transplanted into the neurogenic region of the SVZ, they can differentiate into neurons (Lie et al. 2002), clearly demonstrating that these cells hold a neurogenic potential and that the local microenvironment is crucial for determining the differentiation of these cells. Yet, the modification of the microenvironment may result in proliferation of DAergic neurons in the SN. Functional recovery would be dependent on restoration of the nigrostriatal pathway, which is the next major challenge before this approach could be used as a therapeutic intervention.

Voluntary physical exercise has been shown to have both neuroprotective and proliferative effects in rodent models of PD, and has been associated with the up-regulation of various trophic factors (reviewed in Smith and Zigmond 2003). For example, environmental enrichment and daily physical exercise increased the rate of glial proliferation in the SN of rats with a 6-OHDA lesion, and was accompanied by long lasting improvements in motor function (Steiner et al. 2006). In addition, intense motor therapy targeting limbs affected by the nigrostriatal degeneration in rats with a 6-OHDA lesion, resulted in sparing of DAergic neurons in the SN as well as improvement in motor performance (Tillerson et al. 2001). These findings suggest that daily physical exercise induces cellular plasticity in the SN, possibly through the neurotrophic action of growth factors, and exerts a functional impact on the motor deficits seen after a 6-OHDA lesion.

Successful therapy using endogenous stem cells offers several potential advantages over other strategies of cell-based therapies, but, there is a need of basic research to elucidate the full potential of such therapies. However, exercise represents a non-invasive therapeutic option for PD patients and has proven beneficial for both motor and cognitive dysfunction in several clinical trials, most likely via up-regulation of neurotrophic factors stimulating endogenous repair mechanisms (reviewed in Alonso-Frech et al. 2011; Quinn et al. 2010).

1.1.5 Conclusion and Clinical Trials

PD patients (for a review see: Dunnett 2010; Lindvall 2010) have already been successfully grafted using primary VM tissue in open-label clinical trials, with measurable functional benefits (Mendez et al. 1996; Lindvall 1997; Kordower et al. 1998; Piccini et al. 1999; Winkler et al. 2005). However, negative graft-related side effects were also evident to different extents in some of the transplanted PD subjects, in the form of graft-induced abnormal involuntary movements or dyskinesia (see Lane et al. 2010). These side effects resulted in a return to pre-clinical research in order to elucidate the mechanisms behind this phenomenon. Now, 10–15 years later and following data of promising preclinical studies, clinical trials have been rescheduled for the coming years. TransEuro, a large European multicentre consortium, are currently recruiting patients across the UK, France, Germany and Sweden, who will be transplanted with primary VM-derived tissue in 2012. Alongside this, the consortium has been resolving issues regarding the preparation, storage and transportation of primary tissue, to make it a more viable source of cells. This is a promising step forward for treating PD patients, and will certainly pave the way for this novel neurorestorative approach.

1.2 Huntington's Disease

Huntington's disease is an autosomal dominant neurodegenerative disorder which is characterised both by neuropathological changes, including impaired synaptic plasticity, cortical and subcortical cell loss and the development of interneuronal inclusion pathology, and by behavioural changes, such as marked motor complications and progressive neuropsychiatric and cognitive dysfunction. Underlying these diverse neuropathological and behavioural impairments is a simple genetic mutation, in which the expansion of a CAG triplet repeat exists within the gene on chromosome 4 that codes for the Huntingtin protein (Jones and Hughes 2011). Despite explicit identification of the genetic origin of the progressive degenerative disorder, only a few symptom-modifying interventions are available at present and no treatments exist that are able to cure or slow the inevitable progression of the disease. Since deterioration of the GABAergic medium spiny neurons of the neostriatum constitutes a primary pathological feature of the disease, one strategy aimed at halting the steady development of motor and cognitive impairments has been to replace neurons lost within the cortico-striatal regions, and thereby repair the neural circuitry. The majority of studies to date have evaluated the functional efficacy of grafting the ganglionic eminence (GE), which contains cells fated to develop into the striatum, from primary foetal tissue. A recent exploration of the feasibility of utilising stem cell-derived cell sources has emerged, as well as using stimulation of endogenous stem cells to promote repair via the induction of innate neurogenesis.

1.2.1 Primary Foetal Cells

Since the 1980s, transplantation of the foetal GE into the excitotoxically lesioned striatum of the rat, as a model of HD, has been shown to develop into tissue expressing neurotransmitter- and receptor-related markers characteristic of normal striatal neural tissue (Isacson et al. 1984, 1985, 1987; Graybiel et al. 1987). These transplants are homotopic (i.e. the cells are placed into their natural location), unlike the heterotopic graft method adopted in PD, and therefore have the potential to truly reconstruct circuitry. However, striatal grafts could influence neural function and behaviour through a variety of other mechanisms, including the release of trophic and pharmacological factors, alongside true circuit reconstruction. The demonstration of the integration and functionality of the transplanted cells at the synaptic and systems levels is, therefore, important to truly demonstrate cell replacement. Indeed, striatal grafts have been shown to integrate into the host tissue, as evidenced by the receipt of efferent nigrostriatal, corticostriatal and thalamostriatal connections (McGeer et al. 1984; Pritzel et al. 1986; Rutherford et al. 1987), as well as demonstrating outgrowth to the globus pallidus and SN and forming normal synaptic connections with the host brain (Pritzel et al. 1986; Wictorin et al. 1990). A functional reciprocal interaction between the host brain

and grafted tissue has been demonstrated using the in vivo push–pull perfusion technique to monitor GABA release (Sirinathsinghji et al. 1988). While excitotoxic lesions significantly reduced GABA release in the globus pallidus and SN, foetal GE grafts were shown to substantially restore the levels of release of GABA in these regions and peripherally injected DAergic stimulants were found to even further enhance the GABA release in these regions in grafted rats, in contrast to ungrafted control rats (Sirinathsinghji et al. 1988). These data support the view that foetal GE grafts can integrate functionally into the host tissue and form reciprocal connections with neuroanatomically relevant striatal output areas.

Further support for the functional connectivity of the grafts at the synaptic level has been demonstrated using electrophysiological and neurochemical assays. There exists considerable evidence that grafted striatal neurons display electrophysiological properties similar to the endogenous cell population, that specific patterned signals can be transmitted from host to grafted cells and that transplanted neurons are responsive after stimulation of the host cortical tissue (Rutherford et al. 1987; Walsh et al. 1988; Xu et al. 1991; Siviy et al. 1993). Functional synaptic connections between embryonic striatal grafts and host tissue were demonstrated in the excitotoxic lesion model, and this restitution of the striatal circuitry was shown to result in restored stable synaptic transmission and bi-direction host-graft plasticity that resembled normal circuitry function within the cortico-striatal pathway (Mazzocchi-Jones et al. 2009). Thus, both host-graft synaptic transmission and functional integration have been demonstrated.

The ability of GE grafts to integrate and at least partially repair the neural circuitry disrupted by the lesion is further reflected in the functional efficacy of the grafts. While excitotoxic lesions of the striatum result in marked motor and cognitive impairments, restitution of the neural system and alleviation of behavioural deficits has been demonstrated as a result of transplanting primary foetal neurons. In accordance with this, motor function, as evaluated using the staircase test of skilled forelimb use, can be rescued after implantation of GE grafts in the striatum and the amount of motor recovery has been shown to correlate with both the volume of the graft, as well as with the level of D1 and D2 receptor binding observed via positron omission tomography (Fricker et al. 1997). The learning of motor skills and habits has been associated with corticostriatal function, as evidenced by excitotoxic lesion of this pathway and the consequent loss of function, and the transplantation of the foetal GE has revealed several interesting phenomena. It has been demonstrated that simple replacement of the striatal neurons is not sufficient to the recover the lesion-induced behavioural impairment, but rather targeted retraining of a specific skill is necessary for grafted rats to ‘use the transplant’ and re-learn the lost function (Brasted et al. 2000).

1.2.2 Neural and Embryonic Stem Cells

NSCs have the potential to develop into a range of neural and non-neural cells (Bjornson et al. 1999; Clarke et al. 2000). Thus, in the context of neural repair, functional recovery may be induced due to the multipotentiality of cells, which

allows them to turn into a broad spectrum of neurons and glia, and in turn makes repair of the lost host circuitry possible. In accordance with this, it has been shown that adult rat NSCs that have been immortalised, can develop functional glutamatergic and GABAergic synapses in vitro (Toda et al. 2000). Functional recovery after NSC grafts has also been suggested to occur due to the ability of the cells to induce plasticity in the brain or to provide necessary trophic support. Roberts and colleagues (Roberts et al. 2007) demonstrated that the subset of rats with 3-nitroproprionic acid-induced striatal lesions that were subsequently grafted with NSC transplants, derived from adult hippocampus, exhibited both behavioural performance similar to control rats, as well as normal striatal signal transmission as measured by pharmacological magnetic resonance imaging. Furthermore, it has been suggested that modest behavioural recovery and protection observed on tests of motor function after NSC transplant in a rat model of HD can be associated with the ability of the grafted NSCs to preserve the striatal tissue and slow degeneration, rather than to reduce the lesion extent per se (Roberts et al. 2006). Indeed, more recent enhancement of adult NSCs in vitro has demonstrated an increased ability to generate DARPP-32 expressing striatal-like cells (El-Akabawy et al. 2011), although the yield is still considerably less than can be generated by primary foetal-derived or ES-derived cells.

More recently efforts to repair the damaged neural circuitry in HD have focused on the generation of ESCs for grafting. These are differentiated towards a medium spiny neuron phenotype, the main striatal neuronal population affected by HD, before being grafted into the degenerated brain. While use of these more novel cell lines has obvious advantages, including the ability to produce large numbers of cells at a specified time, thereby reducing logistical and variability-related issues associated with foetal tissue, the disadvantages associated with ESCs (e.g. propensity for proliferation, the specificity of the phenotype) remain to be resolved. Indeed some early attempts at differentiating ESCs resulted in a promising, but marginal yield of DARPP-32 (a marker of medium spiny neurons) expressing cells ($\sim$10 %) in vitro and typically resulted in neural overgrowth in vivo (Aubry et al. 2008; Shin et al. 2011). Nevertheless, considerable advances have been made as of late to generate medium spiny neuron-like cells in vitro from ES-derived cell lines. In a recent study by Ma and colleagues (2012), a modified monolayer differentiation protocol was applied to human ESCs to pattern them into cells characteristic of the lateral ganglionic eminence. Further differentiation of these cells resulted in a yield of 70–80 % GABAergic cells in vitro that expressed markers characteristic of mature medium spiny neurons. When these forebrain patterned progenitors were grafted into mice with excitotoxic lesions and compared with other caudalised progenitors, the forebrain cells differentiated into DARPP-32 expressing cells and the proportion of striatal-like cells (>50 %) was markedly higher than previous findings. Most notably, efferent and afferent connections were established between the striatal-like cells and the host tissue, no overgrowth or tumour formation was evident, and alleviation of motor deficits on a range of tasks was demonstrated (Ma et al. 2012).

The work on ESCs will hopefully pave the way for successful experimental studies with iPSCs, as has been reported recently in PD and stroke. However, experimental injection of other types of stem cells, such as multipotent mesenchymal stromal cells (MSCs), has been conducted in models of HD. Intracerebral injection of MSCs has been shown to induce the proliferation, migration and differentiation of endogenous cells when placed in the dentate gyrus (Munoz et al. 2005). In accordance with this, Snyder and colleagues (Snyder et al. 2010) report that the delivery of human MSCs into the striatum of a transgenic model of HD (N171-82Q HD mice) resulted in increased proliferation and differentiation of endogenous cells for up to a month post-transplant, decreased striatal atrophy and enhanced neurotrophic release, despite the MSCs not being detectable from 3 to 15 days after injection.

1.2.3 Endogenous Neurogenesis

Since an endogenous population of adult neural stem cells will normally proliferate in response to environmental or pharmacological insult or injury, it has been hypothesised that harnessing this innate mechanism may aid in slowing the progressive cell loss evident in HD. Stimulation of endogenous neural stem cells can be induced via multiple routes, including targeted exposure to growth factors and environmental enrichment, and it may constitute a therapy in its own right or work to further enhance the efficacy of other interventions, including cell replacement therapies. In HD, it has been suggested that the machinery responsible for the proliferation and migration of endogenous stem cells is already disrupted as a result of the disease process, resulting in increased neurogenesis in the subventricular zone of patients (Curtis et al. 2003). Interestingly, rodent models of HD typically present with decreased proliferation and differentiation of progenitor cells in the dentate gyrus, but normal subventricular neurogenesis (Gil et al. 2004, 2005; Lazic et al. 2006; Kandasamy et al. 2010; Simpson et al. 2011). While the discrepancy between the rodent models and the human condition are not clear, it has been suggested that the cell cycle marker used to identify proliferating cells in the human HD brain, proliferating cell nuclear antigen, may also be present in cells undergoing DNA repair, making further verification of the functional profile of neurogenetic mechanisms in the human HD brain necessary (Gil-Mohapel et al. 2011). Nevertheless, the therapeutic strategy of stimulating endogenous stem cells remains viable in HD, and proof-of-principle studies conducted in mouse models of HD have demonstrated interesting results.

Environmental enrichment, which can incorporate a range of interventions including adaption of inanimate objects in an area to enhance stimulation and increased social interaction, is known to increase neurogenesis in the adult brain (Rosenzweig et al. 1962; Kempermann et al. 1997). Indeed, environmental enrichment has been reported to slow the disease progression in transgenic mouse models of HD. For example, in R6/2 and R6/1 mice the onset of the motor symptoms was found to be delayed, the clasping phenotype was less pronounced, body weight was

maintained and the peristriatal cerebral volume was less reduced (van Dellen et al. 2000; Spires et al. 2004). The onset of cognitive impairments has also been shown to be delayed after exposure to environmental enrichment, and short- and long-term memory function improved (Nithianantharajah et al. 2009). The beneficial effects of this intervention have been associated with enhanced neurogenesis in HD mouse models, particularly in the dentate gyrus. Lazic and colleagues (Lazic et al. 2006) report an increased number of newly generated neurons surviving and differentiating in the hippocampus, but no change in SVZ neurogenesis. Furthermore, the striatal brain-derived neurotrophic factor (BDNF) deficit observed in non-enriched R6/1 mice can be rescued as a result of environmental enrichment (Spires et al. 2004). Interestingly, unlike enrichment paradigms, physical exercise, such as wheel running, has not been shown to induce the same level of neurophysiological and behavioural benefit. While some alleviation of motor and cognitive impairment is reported after voluntary exercise regimes (Pang et al. 2006; van Dellen et al. 2008), the concurrent improvements in BDNF expression, protein aggregation yield and neurogenesis have not been demonstrated (Pang et al. 2006; Kohl et al. 2007; van Dellen et al. 2008). Nevertheless, both neuropathological recovery as well as behavioural benefits are evident in rodent models of HD after environmental enrichment and, in accordance with this, HD patients exposed to a more stimulating and fertile environment have been shown to demonstrate improved physical, mental and social functioning (Sullivan et al. 2001).

Not only can environmental enrichment enhance the function of endogenous striatal cells, a recent demonstration of the impact of this therapeutic intervention on synaptic function of grafted cells revealed further beneficial effects. Mazzocchi-Jones and colleagues (2011) grafted primary foetal GE cells into C57Bl6 mice with unilateral excitotoxic lesions of the striatum that were housed in either enriched or standard environments. Extracellular recording from in vitro striatal slices revealed increased induction of long-term potentiation in the corticostriatal pathways of enriched mice, as compared to non-enriched mice, and increased BDNF was also observed, thereby demonstrating that the beneficial effects of enrichment are capable of impacting upon both endogenous and grafted cell populations.

The application of growth factors, which demonstrate broad neuroprotective and neurogenic properties, has been of considerable interest in the quest for a therapeutic intervention. FGF-2, which can protect striatal neurons after excitotoxic damage (Bjugstad et al. 2001), was administered to R6/2 mice from 8 weeks of age (Jin et al. 2005) with notable results. Cell proliferation increased 150 % in the SVZ and increased migration and differentiation of neurons in the cortex and striatum were observed. Furthermore, administration of FGF-2 also decreased the yield of neuronal inclusions, improved motor coordination and improved survival age (Jin et al. 2005). The combined administration of adenoviral BDNF and adenoviral Noggin (bone morphogenetic protein antagonist) into the ventricles of 4- and 6-week old R6/2 mice was also shown to increase the recruitment and differentiation of neurons to the striatum, improve motor performance and locomotor activity levels, and increase the age of survival (Cho et al. 2007). BDNF has been reported to not only enhance SVZ neurogenesis, but also to impact upon synaptic mechanisms within the hippocampus.

Impaired synaptic plasticity has been reported in several HD mouse models (Lynch et al. 2007; Simmons et al. 2009) and stable long-term potentiation and improved memory function have been reported after administration or endogenous up-regulation of BNDF (Lynch et al. 2007; Simmons et al. 2009).

Several alternative methods of inducing neurogenesis exist, including administration of antidepressants and the application of cytokines. While the effectiveness of such interventions has been questioned in HD patients (Como et al. 1997; Chari et al. 2003), multiple beneficial effects of selective serotonin re-uptake inhibitors have been reported in HD mouse models on motor and cognitive performance, on BDNF levels and on hippocampal and SVZ neurogenesis (Duan et al. 2004, 2008; Grote et al. 2005; Peng et al. 2008, 2009). Despite previous evidence of having neuroprotective and neurogenic effects, the administration of the cytokine, asialoerythropoietin, in R6/2 mice was ineffective at alleviating behavioural and neuropathological symptoms associated with the disease onset (Gil et al. 2004).

1.2.4 Conclusions and Clinical Trials

While considerable data has been collected from lesion and genetically modified rodent models of HD, translation of the findings into patients is somewhat less advanced. Given the relative infancy of developing striatal-like cells from stem cell sources, the majority of clinical trials performed to date have utilised primary foetal tissue, predominately dissected ganglionic eminence, as the transplantation medium for HD patients. A number of early safety studies were conducted and the results indicated effective survival of cells in the absence of overt side effects, but minimal data regarding the functional efficacy was obtained, making interpretation of these studies difficult (Sramka et al. 1992; Madrazo et al. 1995; Philpott et al. 1997; Kopyov et al. 1998; Ross et al. 1999). A major study initiated in Créteil, France was the first to implement the Core Assessment Protocol for Intracerebral Transplantation in Huntington's Disease (CAPIT-HD) system, a standardised longitudinal assessment battery with specified pre- and post-surgery assessment intervals aimed at identifying neuropsychological and neuropsychiatric changes and using imaging to track neurological alterations. The results of this open-label trial were relatively positive in terms of graft survival and some indication of associated motor and cognitive improvement or stabilization in three out of five patients (Bachoud-Levi et al. 2000; Gaura et al. 2004), although a second open-label trial was less successful, with little evidence of alleviation of deficits and surgical complications during surgery, despite evidence of surviving grafts (Freeman et al. 2000; Hauser et al. 2002). The most promising results to date are those from the French trials, which demonstrate some efficacy of the grafts in terms of both clinical outcome and neurological function, even up to 6 years post-transplant (Bachoud-Levi et al. 2000, 2006; Reuter et al. 2008). Thus, while proof-of-principle has been demonstrated in terms of the potential efficacy of cell replacement therapy in HD, consistent and robust alleviation or stabilization still needs to be further demonstrated in clinical trials. Given that improved physical,

mental and social functioning have been reported in HD patients after exposure to a more stimulating and fertile environment (Sullivan et al. 2001), it may be that a combinatorial approach would produce the most long-term benefit and slowing of disease progression for HD patients (Döbrössy et al. 2010).

1.3 Stroke

Stroke is one of the leading causes of mortality, and the leading cause of disability in the western world. Despite its huge burden on society, currently the only treatment for ischaemic stroke is thrombolysis, which is only suitable for approximately 15 % of patients, and reports in the UK suggest only 1 % receive this treatment (National Sentinel Stroke Audit Phase II 2008). This has lead researchers to consider the possibility of neurorestorative therapies, including cell replacement, enhancement of endogenous neurogenesis and cell therapies which act on the surviving tissue, increasing plasticity and recovery. The field of cell therapy in stroke forms three distinct but not strictly independent camps: cell replacement therapy—where the aim is to replace the missing cells, neuroplasticity/inflammatory modulation—where the stems cells exert modulatory effects on the on-going post-stroke plasticity, inflammation and remodelling, and finally boosting endogenous stem cell proliferation—as both a cell replacement and modulatory strategy. The majority of cell therapies aim to work by two or all three of these mechanisms.

1.3.1 Primary Foetal Cells

Stroke provides more challenges to the possibility of cell replacement therapies than the diseases discussed previously. Unlike HD and PD, the site and degree of cell loss within a group of stroke patients is markedly heterogeneous. This is coupled with the challenge of restoring function due to the loss of multiple cell types, as opposed to the single neuronal subtypes that are targeted in HD and PD. These two points require the careful selection of patient population and cell types for transplantation therapies. The third point may be a larger obstacle for cell replacement therapy, the hostile post-stroke environment. Following an ischaemic event, there are a number of toxic processes which occur at different time scales, including excitotoxicity, necrosis, inflammation and apoptosis, and may ultimately result in the formation of a cyst (for a review see Dirnagl et al. 1999), making the environment potentially hostile to developing cells. For example, inflammation has been demonstrated to be detrimental to newly formed neurons at some time points (Ekdahl et al. 2003), but it can also be beneficial (Ekdahl et al. 2009); therefore, the time point of intervention post-stroke is a crucial factor in ensuring cell survival.

It is clear that in comparison to HD and PD, very little work has been devoted to the study of primary foetal transplants to replace lost tissue following ischaemic stroke, possible due to the issues outlined above. Although initial studies showed that both striatal (Nishino et al. 1993; Aihara et al. 1994) and cortical (Mampalam et al. 1988; Grabowski et al. 1992; Hadani et al. 1992) primary foetal tissue transplants survived in rodent models of focal ischaemia, and that foetal neocortical tissue into cortical infarcts formed limited afferent and efferent connections with the host brain (Grabowski et al. 1992; Sorensen et al. 1996; Dahlqvist et al. 1999), functional improvement due to these grafts was not seen (Grabowski et al. 1996) unless rats were also housed in an enriched environment (Mattsson et al. 1997). Primary striatal transplants appeared to be more successful, with improvements on both motor tests and restoration of GABA levels within the globus pallidus (Nishino et al. 1993; Aihara et al. 1994). More recently, the migration and integration of medium ganglionic eminence transplants in a model of focal ischaemia was demonstrated. An increase in the density of synaptophysin staining was seen in the transplanted animals that had undergone focal ischaemia, when compared to non-treated stroke animals and those treated with fibroblasts as a control. The rats also showed improvement on motor tests, including walking on a rotating beam (rotarod) 1 month following transplantation. These engrafted cells formed synapses with the host tissue, demonstrated on both electrophysiological and structural electron microscopy levels (Daadi et al. 2009).

However, despite some initially promising findings, primary foetal transplants have largely been ignored in the stroke field, in contrast to the HD and PD where they are the gold standard by which other cell replacement therapies are assessed. This is likely related to both the unique challenges posed by stroke and the logistical and technical issues with providing this cell source as a treatment strategy for such a large patient population for whom rapid intervention following insult may be more critical. With the advent of stems cells, the field has grown exponentially and recent focus has been on multiple alterative cells sources, such as bone marrow stromal cells, and with less invasive routes of administration being examined.

1.3.2 Neural and Embryonic Stem Cells

Human NSCs derived from cortex and striatum of foetal tissue and immortalised, have been created by Zaal Kokaia and Olle Lindvall's group. These cells migrate to the site of injury, as has been shown with other foetal-derived NSCs (Kelly et al. 2004), and form neurons on the edges of the transplant. However, cells in the core of the graft 1 month after transplantation were undifferentiated, but despite this, less than 1 % exhibited markers of proliferation, and no tumourigenesis was reported (Darsalia et al. 2007). Interestingly, the striatal derived NSCs migrated further and occupied more space within the striatum than the cortical derived NSCs, indicating that the source of cells affects their final phenotype and migratory potential, as was described with DAergic NSCs for PD. Many studies have

demonstrated that recovery induced by immortalised human NSCs in the ischaemic brain may be not be via neuronal replacement. One study in Gary Steinberg's laboratory demonstrated that foetal-derived human NSCs can increase blood–brain barrier (BBB) integrity, increase expression of proteins involved in tight junctions, increase vascularisation and reduce inflammatory markers post-ischaemia (Horie et al. 2011). This, combined with evidence that the cells induce behavioural improvements on somatosensory tasks prior to migrating to the infarct, indicates that cell replacement is not their only method of action. It has been demonstrated that vascular endothelial growth factor (VEGF) is required for functional recovery induced by these NSC, and blocking VEGF released from the transplanted cells, but not the host, prevents the increased vascularisation, modulation of inflammatory markers and increased expression of proteins related to BBB integrity, usually seen follow transplantation of NSCs. However, increase in BBB integrity was still evident, showing that VEGF is not the only mechanism via which the NSCs are working (Horie et al. 2011). Alongside immortalisation of foetal cell lines, adult subventricular zone cells can be dissected and expanded in culture. Bacigaluppi et al. (2009) report functional recovery with this cell source following systemic administration in a model of focal ischaemia. However, very little differentiation into neurons was evident and the authors link the functional improvement to modulation of inflammatory processes.

Intracerebral transplantation of human ESCs, differentiated into NSCs, into rodent models of focal ischaemia has had similar results to the immortalised human NSCs. Studies have demonstrated that these cells not only survive, but neurons derived from these lines integrate into the host brain, form synapses, exhibit the electrophysiological properties of neurons and elicit functional improvements (Bühnemann et al. 2006; Daadi et al. 2008). Functional benefits have been reported after human ES-derived cells were transplanted 3 weeks post-ischaemia with a matrix scaffold to give the cells structural support within the cyst (Jin et al. 2010a). Apart from this cell source being a promising source for cell replacement, it has also been shown that these cells can increase endogenous neurogenesis and angiogenesis (Jin et al. 2011; Zhang et al. 2011). However, as has been discussed in previous sections, care needs to be taken with the pre-implantation differentiation protocol used on ES cell lines to prevent transplantation of undifferentiated cells and teratoma formation (Erdö et al. 2003; Fong et al. 2007).

1.3.3 Induced Pluripotent Stem Cells

As described previously, the use of autologous cells is advantageous and, in the context of stroke, their use is possibly more promising than in HD or PD, as potential genetic deficits will/could be expressed in cells derived from patients within the latter two disorders. After initially disappointing outcomes with iPSCs in stroke, including the formation of tumours in mice (Kawai et al. 2010), it has been demonstrated that iPSCs can migrate to the site of injury and form neurons

(Jensen et al. 2011). Some of the initial studies also demonstrated amelioration of deficits post-transplantation (Chen et al. 2010; Jiang et al. 2011). However, the most convincing evidence for the potential benefits of iPSCs comes from two recent studies. iPSCs derived from human fibroblasts differentiated into a neural phenotype (neuroepithelial-like stem cells) formed neuronal populations following intra-striatal transplantation into focal stroke models, with only 1 % of grafted cells expressing proliferating markers 4 months post-grafting and no tumour formation (Oki et al. 2012). These striatal grafts formed connections with the host globus pallidus and functional synapses. Functional restoration of skilled reaching behaviour was evident 1 week post-transplantation, prior to the formation of neurons, and the authors suggest that one mechanism for this improvement may be related to the increased VEGF immunoreactivity evident following transplantation, and its influence on the post-stroke brain as described above (for a review see Hermann and Zechariah 2009). A second study using another iPSC source, demonstrated a reversal in somatosensory deficits 2 weeks post-transplantation. However, development of GABAergic DARPP-32 expressing neurons from the grafted iPSCs took several months, but once developed these cells formed projections to the SN. This correlated with other behavioural improvements. Transplantation of the iPSCs also protected the SN from secondary degeneration due to loss of neurons in the striatum (Onteniente 2012, personal communication).

These two studies highlight that iPSCs may be functioning via two mechanisms in the ischaemic brain: initially by modulating the on-going inflammation, angiogenesis and plasticity events and secondly via functional reconstruction of the circuitry. In order to see the second effect, cells need to be left in situ for long periods, as development of specific neuronal populations can take more than a month, and functional connectivity, particularly projections to distant structures such as the SN, can take many months. This is an issue commonly acknowledged when examining striatal transplantation in the HD field, but long-term follow-up of animals is not commonly seen in the stroke literature.

1.3.4 Marrow Stromal Cells

Bone-marrow derived stromal cells are a mixed cell population that includes stem cells and progenitor cells, which are extracted from bone marrow and, as with iPSCs, allow for autologous transplantation, negating issues of immune rejection. This source includes hematopoietic stem cells, mesenchymal stem cells, endothelial progenitors and very small embryonic-like cells. The mix of these cells is commonly called mesenchymal stem cells ('MSCs'), although this term should be reserved for a specific subpopulation of these cells, which are identified as plastic-adherent fibroblastic cells that express, in humans, CD105 (SH2), SH3, Stro-1, and CD13 but not CD34 and CD45.

MSCs migrate to areas of inflammation and, due to the blood–brain barrier disruption following stroke, they can be transplanted via multiple routes—intracerebral, intraventricular, intracisternal, intravenous—and still reach the brain to

improve neurological outcome (for review see: Li and Chopp 2009). MSCs are also endogenously recruited from the bone marrow following stress and induction of cytokines (for a review see: Lapidot and Kollet 2010), and preclinical studies have demonstrated functional benefit in models of stroke from increasing this endogenous effect using granulocyte-colony stimulating factor (for review see: England et al. 2009), which has led to clinical trials demonstrating the safety of this treatment in stroke patients (Schäbitz et al. 2010; Boy et al. 2011; Floel et al. 2011; England et al. 2012) but, the latest "AXIS 2" trial failed to show efficacy (Sygnis 2011). When examining exogenous transplantation of this cell source, no matter the route of administration, the cells selectively target the damage and this is achieved via the stromal-derived factor-1 chemokine and the CXCR4 receptor expressed on the MSCs (Cui et al. 2007).

MSCs possess the ability to differentiate into mesodermal cell lineages, and when transplanted these cells most probably do not exert their functional effects via replacement of neurons per se. For example, one study showed that 1 year after transarterial transplantation only a relatively small number of the transplanted cells were present in the brain (but more than other organs examined), and only a small percentage of these expressed neuronal markers (Shen et al. 2007). The existence of a neuronal phenotype can be increased by transfecting the cells with Notch intracellular domain and then differentiating them to a neural phenotype prior to transplantation, producing increased improvement on functional outcome measures compared to normal MSCs (Hayase et al. 2009). Despite the low survival and neuronal differentiation, MSCs do secrete a number of factors which have been implicated in their beneficial effect post-stroke (e.g. BDNF, VEGF, Hepatocyte growth factor, nerve growth factor, basic fibroblast growth factor and insulin growth factor-1 (for a review see: Li and Chopp 2009)). Not only do these cells secrete beneficial factors, the ischaemic environment alters the gene expression profile of these cells to increase this expression. Microarray analysis of MSCs grown in a media containing extracted supernatant from an ischaemic brain, showed increased expression of FGF2, insulin-like growth factor 1, VEGFa, nerve growth factor beta (bNGF), BDNF and epidermal growth factor (Qu et al. 2007). Apart from MSCs secreting neurotrophic factors themselves, there is also evidence to indicate that these cells act on astrocytes, stimulating them to produce beneficial factors. Co-cultures of MSCs and astrocytes demonstrate that MSCs promote BDNF, VEGF and bFGF expression within the astrocyte population (Gao et al. 2005). MSCs also increase expression of bone morphogenetic protein 2/bone morphogenetic protein 4 and connexin-43 in astrocytes, which are involved in gap junction formation and intercellular channels within astrocytes, indicating that MSCs are potentially remodelling the astrocyte network (Zhang et al. 2006). Not all of these effects are orchestrated by the secretion of growth factors. Recently, the role of microRNA has been implicated in the communication between MSCs and host brain tissue (Xin et al. 2012).

It is believed therefore that the primary functional effects of MSCs is via their secretion of growth factors which in turn act on the host brain, which in turn exerts beneficial effects on the ischaemic environment. This includes reducing apoptotic

cell death (Chen et al. 2003a), promoting glial remodelling (Li et al. 2005), vascular remodelling (Chen et al. 2003b), decreasing BBB leakage (Zacharek et al. 2007), increasing levels of anti-inflammatory cytokines (Liu et al. 2009; Li et al. 2010) and enhancing endogenous neurogenesis (Li et al. 2002; Chen et al. 2003a). There is also evidence that this cell source effects neuronal plasticity by reducing the glial scar that is formed around the infarct (Li et al. 2005; Shen et al. 2006), increasing axonal sprouting (Andrews et al. 2008) and synaptophysin expression (Shen et al. 2006). MSCs appear to be increasing the levels of brain plasticity and remodelling that occur naturally post-stroke. Interestingly, their effects are not limited to the brain as MSCs also induce axonal outgrowth in the spinal cord (Liu et al. 2007). While this is a region of the CNS that is not commonly examined post-stroke, damage to the internal capsule fibres will cause destruction of parts of the cortico–spinal tract, resulting in degeneration in the spinal cord.

Unlike many other pharmacological neuroprotectants examined in models of focal ischaemia, cells derived from bone marrow have been demonstrated to be effective even if transplanted 1 month following the ischaemic event (Shen et al. 2007; Yasuhara et al. 2009) and one study has demonstrated their beneficial effects are still evident 1 year post-transplantation (Shen et al. 2007). One point that should be highlighted in preclinical stroke research is the importance of testing potential therapeutics in animal models that express the co-morbidities seen in the human population of stroke patients, such as hypertension, diabetes, and ageing. This is to ensure not only that the treatments will be beneficial in patients expressing these co-morbidities, but that they will be safe. A notable example of the impact of these interacting factors was observed when MSCs were tested in diabetic rats and they were found to increase blood–brain barrier leakage, increase incidence of haemorrhage and result in greater mortality (Chen et al. 2011), indicating that diabetes may be a contraindication for this cell source.

1.3.5 Endogenous Neurogenesis

Following an ischaemic stroke there are a number of beneficial endogenous processes that arise, including on-going neuronal plasticity (for a review see: Benowitz and Carmichael 2010) and endogenous neurogenesis, which may lead to degree of recovery that many patients exhibit post-stroke. It has been demonstrated in humans (Minger et al. 2007) and experimental models that ischaemia can stimulate endogenous neurogenesis from both the SGZ and SVZ (Jin et al. 2001; Zhang et al. 2001; Arvidsson et al. 2002). Within the SVZ it has been demonstrated that there is a significant increase in doublecortin-positive neuroblasts following ischaemia, however approximately 80 % of these died before forming mature neurons. The authors also estimated that only 0.2 % of the neurons that died following the ischaemic event were replaced (Arvidsson et al. 2002). However, issues with labelling newly formed cells may have resulted in an underestimation. Despite the low numbers, this is a relatively long-term event that occurs following stroke and doublecortin-positive cells have been shown to be

continuously produced for at least 4 months following ischaemia (Thored et al. 2006). These cells migrate along blood vessels to the site of injury and those that survive can become functional neurons that form synapses with neighbouring cells (Yamashita et al. 2006), and have the electrophysiological properties of neurons (Hou et al. 2008).

The number of neurons produced due to endogenous neurogenesis is low, and their role in post-stroke recovery is not clear. However, it has been demonstrated that ablation of post-stroke neuroblast formation produces larger lesions and an increase in behavioural deficits following ischaemia (Jin et al. 2010b). This effect was evident 24 h following ischaemia, before the neuroblasts could have produced functional neurons, suggesting that endogenous neurogenesis may exert beneficial effects through faster acting mechanisms than cell replacement, potentially by many of the processes described above for other stem cell types.

A number of ways to optimise post-stroke endogenous neurogenesis have been examined, in order to improve functional outcome. As described with both HD and PD, environmental enrichment (Komitova et al. 2005) and exercise (Hicks et al. 2007) have been evaluated and shown to increase endogenous neurogenesis in rodent models of stroke, alongside improving functional outcome. A number of therapeutics have also been examined for their ability to boost endogenous neurogenesis in models of focal ischaemia, for example, growth and neurotrophic factors, including erythropoietin (for a review see: Sirén et al. 2009), meteorin (Wang et al. 2012) and cerebrolysin (Zhang et al. 2010). Interestingly, immunosuppression results in increased endogenous neural and progenitor cell migration. However, despite the immunosuppression inducing a functional benefit, these extra stem cells do not form neurons (Erlandsson et al. 2011). Apart from pharmacological and physical interventions, transplantation of many of the cell sources, as alluded to above, also increase endogenous neurogenesis, thereby combining stimulation of endogenous neurogenesis with exogenous cell transplantation. However, there have also been reports of decreased endogenous neurogenesis following exogenous cell therapy, possibly due to microglial activation (Minnerup et al. 2011).

1.3.6 Conclusion and Clinical Trials

Multiple avenues are being examined towards effective cell therapy for stroke, using a wide range of cell sources, too numerous to cover exhaustively in this chapter (see: Rojin and Verfaillie 2012; Sanberg et al. 2012). It is evident that these stem cells work via multiple routes of action, whether that is replacement of cells, stimulation of endogenous neurogenesis or modulation of the ischaemic environment, and the best hope for a treatment would come from utilising all three.

A number of clinical trials have been carried out using stem cells and cell therapies in stroke (see: Sinden et al. 2012). Intracerebral grafting of human teratocarcinoma cell line NTera-2 (NT-2) was examined in the early 2000s (Kondziolka et al. 2000, 2005; Nelson et al. 2002). This had some, but not robust,

suggestions of efficacy. Following this, transplantation of porcine primary cells was tested (Savitz et al. 2005). This removed some of the logistical issues surrounding supply of primary tissue, but the trial was stopped by the FDA after two of the five patients demonstrated adverse outcomes. Currently, ReNeurone, a UK based company, is conducting a clinical trial transplanting neural stem cells intracerebrally and, although functional benefits of these cells have been seen in rodent models, markers of mature neurones are not evident in the grafts 3 months after transplantation (Stroemer et al. 2009), and little is known about how the cells cause the observed benefit. A separate trial of SanBio's stem cell line SB623 administered intracerebrally is also underway in the US (www.clincialtrials.gov).). There are also several trials examining intravenous and arterial administration of non-neural stem cells, such as MSCs, with some preliminary data emerging suggesting improvements in patient function (Honmou et al. 2011). However, a cautious approach should be taken before progressing to clinical trials. A recent meta-analysis of preclinical testing of stem cell therapy in stroke highlights a significant publication bias and poor quality of study design. This introduces potential confounds to data and reinforces the need to introduce more rigour to preclinical stroke research (Lees et al. 2012). If negative effects arise from clinical trials with cell therapy in stroke, either by demonstrating no clinical improvement or, worse, safety concerns, it would put the whole field back a number of years and take time to re-build the confidence of funders and the public, as was the case with graft-induced dyskinesia in PD. Therefore, a number of issues need to be addressed before a potential cell source should be carried through to the clinic. This includes long-term assessment of the cells in vivo in models of focal ischaemia, utilising models that are clinically relevant, testing potential therapeutics in aged, hypertensive and diabetic animals to ensure the efficacy and safety of the cell lines, ensuring that the cell source will not be tumourigenic, and elucidating more fully the mechanisms by which the cells are impacting upon the host tissue.

2 Conclusion

Transplanted and endogenous stem cells has been investigated to treat multiple CNS disorders, and promising results have been delivered. We have discussed the use of cell-based therapies for Parkinson's, Huntington's Disease and stroke. In all these diseases, there is evidence of exogenously transplanted cells functionally integrating into the host brain, replacing the damaged tissue, and having a behavioural benefit, but this is not the cells only mechanism of action. It appears, particularly in stroke, that stem cells can stimulate endogenous neuroprotective and restorative events, bringing about functional improvements. However, with both Parkinson's and Huntington's disease cell replacement therapy with primary cell transplantation is still the gold standard. Despite this promising therapeutic approach, there is still a need to develop novel cell sources for all CNS disorders

that are more abundantly available, and if possible, negate the issues of rejection evident with non-autologus cell transplants, removing the requirement for patients to be given immunosuppression. This is especially important in stroke, where patients are often already suffering from pathological immunodepression. Therefore, iPS cells are a hopeful alternative.

Stimulation of endogenous neurogenesis would be the ideal treatment for patients, boosting the natural neurogenic processes in the brain. It is unlikely that this alone will provide the cell numbers required to treat these aggressive and devastating disorders. Therefore, a combination of cell replacement and stimulation of endogenous events, such as neurogenesis, is likely to provide the best hope for patients. However, as stated above, we need to proceed with caution. For all these CNS disorders, novel cell sources need to be thoroughly tested prior to moving to clinical trials, using appropriate models, sensitive outcome measures and assessment at relevant time points. If inconclusive or negative results are gained from premature clinical trials, funders and the public will lose faith in the cell therapy field, which will have a negative impact on research for years to come.

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Curr Topics Behav Neurosci (2013) 15: 399–401
DOI: 10.1007/978-3-642-36232-3,