Review Article

Regulation of adult neurogenesis in the hippocampus by stress, acetylcholine and dopamine

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Abstract

Neurogenesis is well-established to occur during adulthood in two regions of the brain, the subventricular zone (SVZ) and the subgranular zone (SGZ) of the dentate gyrus in the hippocampus. Research for more than two decades has implicated a role for adult neurogenesis in several brain functions including learning and effects of antidepressants and antipsychotics. Clear understanding of the players involved in the regulation of adult neurogenesis is emerging. We review evidence for the role of stress, dopamine (DA) and acetylcholine (ACh) as regulators of neurogenesis in the SGZ. Largely, stress decreases neurogenesis, while the effects of ACh and DA depend on the type of receptors mediating their action. Increasingly, the new neurons formed in adulthood are potentially linked to crucial brain processes such as learning and memory. In brain disorders like Alzheimer and Parkinson disease, stress-induced cognitive dysfunction, depression and age-associated dementia, the necessity to restore brain functions is enormous. Activation of the resident stem cells in the adult brain to treat neuropsychiatric disorders has immense potential and understanding the mechanisms of regulation of adult neurogenesis by endogenous and exogenous factors holds the key to develop therapeutic strategies for the debilitating neurological and psychiatric disorders.

Key words: Acetylcholine, adult neurogenesis, activation of resident stem cells, Alzheimer disease, dopamine, Parkinson disease, stress

INTRODUCTION

The Spanish histologist, psychologist and Nobel laureate said "Once development was ended, the fonts of growth and regeneration of the axons and dendrites dried up irrevocably. In the adult centers, the nerve paths are something fixed, and immutable: Everything may die, nothing may be regenerated".[1] Until recently, it has been generally believed that the mammalian adult central nervous system (CNS) has a limited regenerative capacity.[2] Further it was considered that the major repair mechanisms in the CNS were post-mitotic, which includes the sprouting of axon terminals, changes in the expression of neurotransmitter-receptors and synaptic reorganization, while replacement of dying/degenerating neurons was not believed to occur.[3] However, more than half a century ago, Altman and Das[4] suggested that neurogenesis continues throughout adulthood, and since then a large body of evidence has demonstrated that new cells that mature to become neurons or glia are indeed born in restricted regions of the adult mammalian CNS.

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Altman and Das[4] for the first time used [^{3}H]-thymidine autoradiography and reported the generation of new neurons in a variety of structures in the adult rat and cat including the olfactory bulb, hippocampus and cerebral cortex. Consequently, Michael Kaplan and co-authors showed [^{3}H]-thymidine-labeled cells in the cerebral cortex, dentate gyrus (DG), olfactory bulb of adult rats and mitosis in the subventricular zone (SVZ) of adult

macaque monkeys by combining [^{3}H]-thymidine labeling and electron microscopy.[5-8] Since then adult neurogenesis has consistently been found in the SVZ of the lateral ventricle[9,10] and in the hippocampal subgranular zone (SGZ).[11-13] Studies have now confirmed that neuron production, migration and differentiation are major developmental events that continue on a smaller scale, into adult life in a wide range of species from insects to mammals.[14] Elizabeth Gould and her colleagues have found new cells with neuronal characteristics in the neocortex of adult rats and macaques[15] consistent with the earlier findings of Altman[4] and Kaplan.[5] Apart from the rodents and macaques, adult neurogenesis is also reported in the DG of tree shrews, marmoset monkeys and humans.[16-20]

The newly generated neurons in the SGZ migrate to the inner granule cell layer, rapidly extend long axonal projections along the mossy fiber pathway and reach their target CA3 pyramidal neuronal cell layer within 4-10 days after division,[21] form connections with the CA3 neurons, hilar interneurons[21] and release glutamate as their main neurotransmitter[22] thus attaining a functional significance. Newly generated cells in the adult mouse hippocampus are found to exhibit neuronal morphology and display passive membrane properties, action potentials and functional synaptic inputs similar to those found in the mature DG cells.[23] New-born granule neurons in the DG appear to first receive GABAergic synaptic inputs around 1 week after birth and then glutamatergic inputs by 2 weeks.[24,25] Young granule neurons appear to have a high input resistance and a sub-threshold Ca^{2+}-conductance, which finally enable action potential firing with very small excitatory currents.[26] The enhanced excitability might be important for the young neurons when only a few excitatory contacts have been formed. Furthermore, new-born neurons exhibit special properties in synaptic plasticity, such as having a lower threshold for the induction of long-term potentiation (LTP)[26,27] than mature neurons. Figure 1 shows the events of neurogenesis in the DG of the hippocampus.

The discoveries of the phenomenon of adult neurogenesis lead to the search for the behavioral consequence of such a process continuing in adulthood. Subsequent to evidence for a positive relationship between adult neurogenesis and learning in the song system of canaries[28] and zebra finches,[29] several studies have looked into a plausible

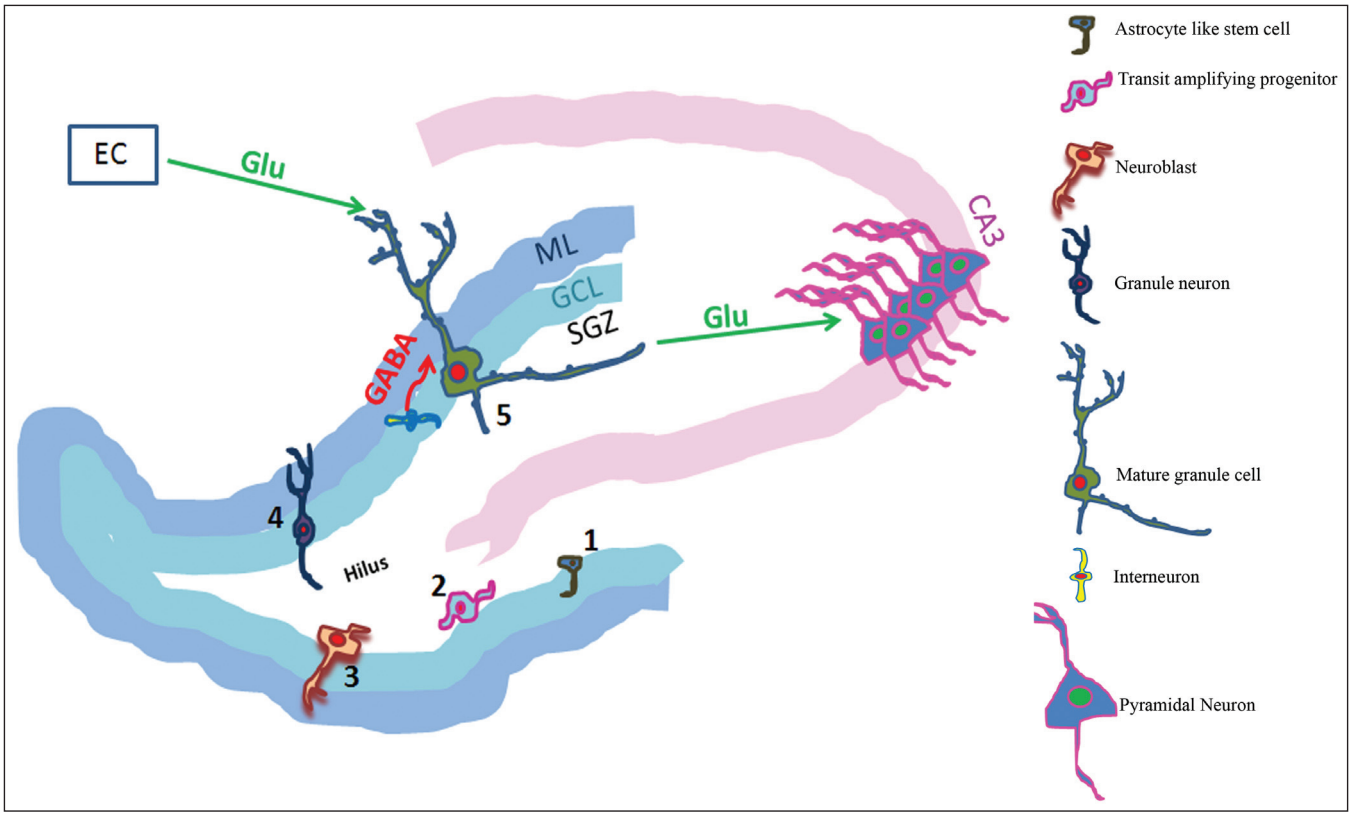

Figure 1: The subgranular zone (SGZ) of the dentate gyrus shelters the astrocyte like stem cells (1), which generate the transit amplifying progenitor cells (2). These transit amplifying cells mature into neuroblasts (3), which migrate (4) in the vicinity into the granule cell layer (GCL) and differentiate into granule neurons (5). These newly generated granule neurons show initial signs of functional interconnections by extending axonal projections along the mossy fiber towards the CA3, and advancing their dendritic branches into the molecular layer (ML). Once these cells mature, they receive glutamatergic (Glu) inputs from the entorhinal cortex (EC), GABAergic inputs from the interneurons and provide glutamatergic inputs to the CA3 neurons.

relationship between learning and adult neurogenesis. Mice with the fewest number of new neurons performed poorly during spatial navigation learning[30] and ablation of neurogenesis by focal irradiation impaired contextual fear conditioning and performance in a hidden platform task,[31,32] while training on associative learning tasks and reactivity to a novel environment enhanced neurogenesis in the adult hippocampus.[19,33] Conditions that increase the number of immature neurons such as estrogen,[34] environmental complexity[35] and physical exercise[36,37] also tend to enhance performance on hippocampal-dependent learning tasks[35,36,38,39] suggesting a potential link between hippocampal neurogenesis and learning. But the issue remains controversial with studies showing that some forms of learning do not recruit adult neurogenesis.[40-43]

METHODOLOGIES FOR INVESTIGATING ADULT NEUROGENESIS

The technical advances in the field of adult neurogenesis have facilitated identification of new-born neurons among the existing neurons in the adult CNS. Four approaches have been explored so far.

Analysis of the incorporation of nucleotide analogs during cell division

During DNA replication in the S-phase of the cell cycle, exogenous nucleotides such as [H^3]-thymidine or bromodeoxyuridine (BrdU) are incorporated into newly synthesized DNA and then passed on to cell progeny. [H3]-thymidine is detected using autoradiography and if the exposure times and development procedures used are consistent, reliable stoichiometry can be achieved. BrdU is more extensively used to detect neurogenesis because of the possibility to detect it using immunohistochemistry (not stoichiometric) and more importantly, because it can be co-localized with histological markers for neurons or glia, thus permitting phenotypic analysis. Figure 2 shows the representative micrograph with BrdU-immunoreactivity co-localized with a neuronal (NeuN) or an astrocytic (S100β) marker. However, nucleotide analogs have some limitations. The first one being the requirement of tissue fixation and DNA denaturation that makes it unsuitable for analyzing live cells.[44] Secondly, after several cycles of cell division, dilution of BrdU to undetectable levels can occur.[45] Thirdly, BrdU or other nuclear markers stain only the cell body, so it cannot be used to analyze the synaptic properties of new-born neurons.[46] In addition we must remember that BrdU or [H^3]-thymidine incorporation is an indication of DNA synthesis only and not cell division. Incorporation of nucleotide analogs also occurs into nicked, damaged DNA undergoing repair also, although on a smaller scale than during DNA replication.[47] Thus the dose and duration of BrdU pulsing, as well as the detection of BrdU, need to be appropriately controlled to avoid misidentification of repairing/dying cells as new-born cells. A few studies have also used iododeoxyuridine (IdU) and chlorodeoxyuridine (CldU), thymidine analogs with a structure similar to BrdU, to label newly generated neurons.[48-52] However, these analogs do not seem to be as sensitive as BrdU in being incorporated into the newly-born cells at doses similar to those of a saturating BrdU dose.[53]

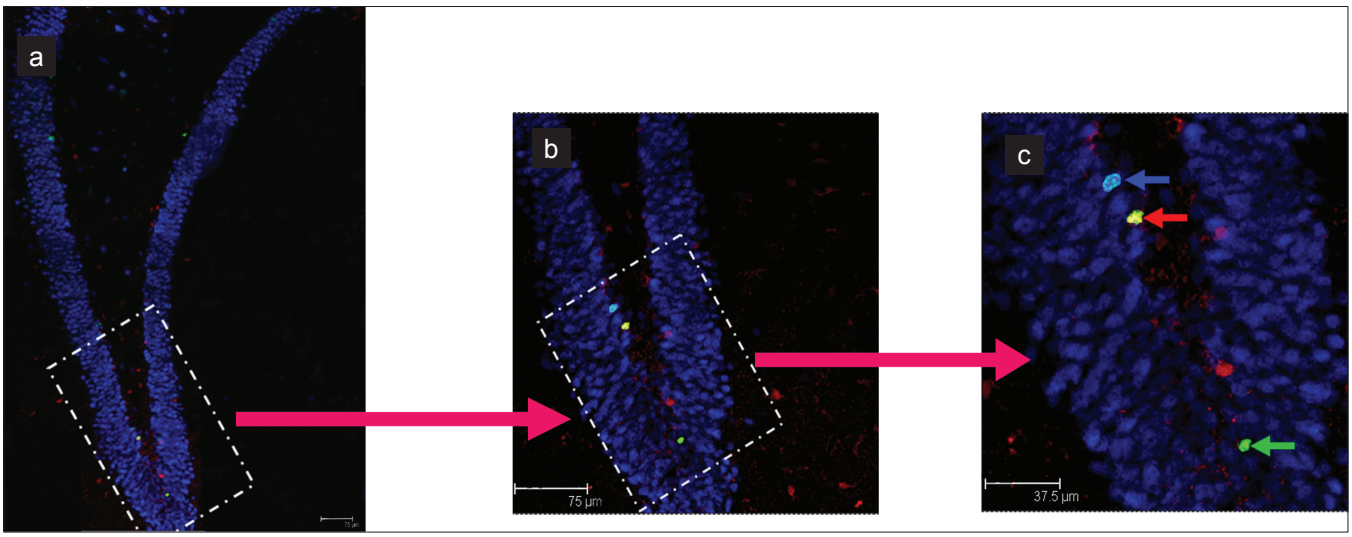

Figure 2: Representative confocal images of triple labeling for differentiation studies. (a) is the reconstructed image of dentate gyrus in the dorsal hippocampus. (b) represents the 4X zoomed image of the region marked in white dotted rectangle in a, and c represents 8X zoomed image of the region marked in b. The blue arrow in c represents the BrdU-ir cell colocalized with NeuN (BrdU+/NeuN+, appears cyan), the red arrow points at the BrdU-ir cell colocalized with S100β (BrdU+/S100β+, appears yellow) and the green arrow indicates a BrdU-ir cell which is not colocalized with either NeuN nor S100β (BrdU+, appears green; potentially undifferentiated). Scale bar = 75µm in a and b, 37.5µm in c.

Thus BrdU has been the most widely– and reliably–used marker for survival and differentiation studies in adult neurogenesis.

Analysis based on expression of specific markers

Developing neurons express distinct markers during their maturation.[54] Proliferating cells are labeled using Ki67, an endogenous marker expressed during most phases of the cell cycle including the S-phase[55,56] or by PCNA (proliferating cell nuclear antigen) that labels cells undergoing division. For immature neurons, PSA-NCAM (poly-sialylated-neural cell-adhesion molecule), Tuj1 (β-tubulin isoform III), CRMP (collaspin response-mediated protein 4, also known as TOAD4), NeuroD and DCX (doublecortin) are commonly used.[23] Among the markers used for mature neurons are MAP-2ab (microtubule associated protein-2 a and b isoforms),[57] NeuN (neuronal nuclei),[11] and Prox-1 (a dentate gyrus cell marker)[58] calbindin[11] and calretinin.[59] New-born neurons can be identified by the presence of immature markers and absence of mature markers of neurons. The requirement for this approach relates to the specificity of the markers since some antibodies to these markers (e.g., Tuj1) also stain non-neuronal cells[60] and some markers (e.g., PSA-NCAM) are re-expressed in pre-existing neurons[61] under certain conditions.

Analysis based on genetic marking with retroviruses

The main drawback with the markers such as BrdU is the inability to perform functional studies. This was overcome by van Praag and colleagues[46] who showed that the new-born cells expressing neuronal markers in the SGZ become functional neurons, using retroviral vector expressing green fluorescent protein (GFP). The use of replication-incompetent retroviruses allow specific labeling of dividing cells and their progeny since they are unable to infect post-mitotic cells, such as neurons.[62] The expression of transgenes from retroviruses requires viral integration into the host genome.[63] For retroviruses that lack nuclear import mechanisms, such as the Muloney murine leukemia virus, viral integration occurs only when the nuclear membrane breaks down during mitosis[63] and serves as a good indicator of cell division. The strategy used to detect neurogenesis is to incorporate the coding sequences of histochemical markers such as GFP into the retroviruses. Since the marker sequence is integrated into the host genome, the problem of dilution (that is seen with BrdU) is eliminated. Retroviruses deliver single copies of the transgenes into their target cells, thus allowing low expression of these proteins since high expression could interfere with the development or function of the synapses.[64] The most important advantage with the retroviral method is that it permits the live imaging of the cells in brain slices allowing the analysis of the structural and functional integration into the host circuitry and also map the physiological maturation of these new-born neurons.[65] The retroviral technique can also be used to knockout specific proteins in the new-born cells, in combination with Cre-loxP technology,[66] or use shRNA[24,67,68] to inhibit expression of the particular protein, only in the labeled cells. However, this method is also not devoid of disadvantages. These retroviruses have to be stereotaxically introduced into the brain and the inflammation due to the injection could alter neurogenesis.[65] Further, fusion of microglia with existing post-mitotic neurons have been reported after combination of retroviruses with microglia.[69] Retroviruses are silenced in certain host cells and the two daughter cells from the same parent cell could undergo differential silencing or could be silenced during differentiation.[70]

Analysis based on fluorescent protein expression under the control of promoter for specific proteins

The generation of transgenic mice expressing fluorescent protein under the control of promoter for specific protein allows visualization and specific manipulation of new-born neurons in the adult CNS with out the disadvantages of stereotaxic surgery and retroviral injection.[71,72] Adult mice expressing GFP under the control of the regulatory regions of the nestin gene reveal both neural progenitors and some immature neurons.[72] With GFP expressed under the transcriptional control of the pro–opiomelanocortin (POMC) genomic sequences, a population of newly-born granule cells of the DG is selectively labeled.[71] In a recent study,[73] GFP expressed under the control of the GAD67 promoter surprisingly labeled only newly-born neurons in the SGZ without labeling the new-born neurons in the SVZ/RMS. These GFP-expressing neurons exhibited histological markers and electrophysiological properties similar to that of immature neurons. Similar to the retroviral technique, this allows the analysis of the morphology of new-born neurons. However, the disadvantage is that most of these markers are expressed only during early maturation.[73,74]

In an interesting breakthrough, a transgenic mouse line with tamoxifen-inducible recombinant CreER^{T2} under the control of BAC-based promoter of DCX has been described.[75,76] In the DCX-CreER mice, the recombination is temporally controlled by the administration of tamoxifen and the BAC-based promoter of DCX spatially restricts it to the new-born neurons. The labeling is permanent once the recombination occurs and the stable fluorescent protein expression permits detailed morphological and electrophysiological analysis.[75]

Thus, more tools are emerging to study neurogenesis, particularly the functional properties of the new-born

neurons. Channelrhodopsin or halorhodopsin could be potentially expressed in a population of the new-born neurons to study the properties of the new-born neurons *in vivo* or how different factors affect neurogenesis.[77]

FACTORS INFLUENCING ADULT NEUROGENESIS

The rate of neurogenesis is modulated by various physiological and pathological conditions. The newly generated cells may have a function in cognition and brain repair mechanisms. Enriched environment and exercise increases neurogenesis and is associated with improved memory functioning and enhanced synaptic plasticity. Several pathological conditions are known to modulate hippocampal neurogenesis. Neurological diseases, stroke and traumatic brain injury favour neurogenesis, which could be aimed at promoting recovery.[78] On the other hand, ageing,[78] social isolation,[79] alcohol consumption,[80] odor deprivation and maternal deprivation stress are found to have negative effects on neurogenesis in the DG.[81] In this review, we highlight the key findings regarding the effects of stress on adult neurogenesis. While considerable research has been done on the regulation of adult neurogenesis by acetylcholine (ACh) and dopamine (DA), we still do not have a comprehensive understanding of their roles. Accordingly, we review the evidence for the regulation of adult neurogenesis by ACh and DA, which also play an important role in learning and memory and is known to be affected by chronic stress and depression.

STRESS AND NEUROGENESIS

The maintenance of homeostasis is one of the key features in normal physiological functioning and stress alters this homeostasis. When stress is severe and prolonged, it can result in impairment in learning and memory and precipitate affective disorders like depression. Chronic exposure of rodents to physical stress or exposure of nonhuman primates to psychosocial stress is reported to cause atrophy of CA3 pyramidal neurons in the hippocampus,[82-88] increase glucocorticoid secretion, activate release of excitatory amino acids, decrease DA levels and increase DA turnover in the hippocampus[89-91] and decrease AChE activity in the hippocampus.[85,89,92]

With respect to hippocampal adult neurogenesis, stress is largely detrimental independent of stressor, species or life stage.[93] Adult neurogenesis is decreased by different types of stressors, including predator odor,[94] social stress,[17,95] acute and chronic restraint stress,[96-100] footshock stress[100,101] and chronic mild stress.[102] It has been observed that different stressors including fox odor, subordination and physical restraint decreases the proliferation and survival of new-born neurons in the adult SGZ of many mammalian species, including rats, tree shrews and marmosets.[15] Proliferation in adult monkeys is diminished in a resident intruder model of stress[18] and in a chronic mild stress model in Wistar rats.[103] Restraint stress of pregnant rats and acoustic startle stress of pregnant macaques produced a lasting suppression of cell proliferation in the DG of the offspring[104,105] and extends into adulthood in rats.[105] Maternal separation in rats during the early post-natal period inhibits cell proliferation and the production of immature neurons in the DG of the adult offspring.[81]

Studies show that stress affects the entire process of neurogenesis. Chronic restraint stress for 3 weeks suppressed cell proliferation[97,98] while acute restraint stress for 2 h did not show any major changes.[96] Rats subjected to chronic or intense uncontrollable stress in adulthood also exhibited prolonged inhibition of cell proliferation in the DG.[101,106,107] By contrast, rats subjected to acute stress in adulthood appear to exhibit a recovery of baseline cell proliferation by the following day.[106] Following chronic stress, increases in the cell cycle inhibitor p27Kip1 parallels the decreased proliferation and apoptosis, indicating that more cells had entered cell cycle arrest and that the granule cell turnover had thus slowed down.[108,109]

Chronic mild stress or chronic restraint stress was shown to decrease survival of new-born cells in adult rat hippocampus.[97,98,110] Acute psychosocial stress diminishes both short-term and long-term survival of newly differentiated DG neurons.[50,111] Further, stress is shown to differentially affect the differentiation of the newly formed cells. While a few studies support suppression in production of new neurons,[95,96,112] others report enhanced survival and unaffected neuronal maturation[94,101] following stress. All these evidences suggest that stress more often than not has a detrimental effect on hippocampal neurogenesis. Table 1 summarizes the effects of different stressors on the different stages of neurogenesis in the adult DG.

Although the cascade of events leading to a reduction in adult neurogenesis in the DG following stress is far from understood, substantial evidence suggests that stress hormones play an important role. Gould *et al*[113] showed that adrenal steroids probably underlie this effect as stress increases adrenal steroid levels and glucocorticoids decrease the rate of neurogenesis.[113] Cameron and McKay extended these findings by showing that the decreased level of neurogenesis in the DG during ageing[11] is due to an increase in glucocorticoid levels and that adrenalectomy could reverse the decline in DG neurogenesis.[114] Increased

Table 1: Summary of the effects of various forms of stress on neurogenesis

Type of stressor	Species	Duration	Effect			References
			Proliferation	Survival	Differentiation	
Exposure to predator odor	Male SD rats	1h	↓	⇔	⇔	[94,164]
Exposure to predator odor	Female SD rats	1h	⇔	–	⇔	[164]
Psychosocial stress	Adult tree shrews	1h	↓	–	–	[17]
Acute psychosocial stress	Male SD rats	1 day	⇔	↓	↓	[50]
Chronic psychosocial stress	Male tree shrews	7+28 days	↓	–	–	[111]
Resident intruder stress	Marmoset monkeys	1h	↓	–	–	[18]
Chronic restraint stress	SD rats	6h per day, 3 weeks	↓	Slight ↓	⇔	[96]
Acute restraint stress	SD rats	2h and 6h single session	⇔	–	–	[96]
Restraint stress	SD rats	4h per day, 7 days	↓	–	–	[107]
Restraint stress	Male SD rats	^{3}h one session	↓	–	–	[165]
Restraint stress	C57BL/6J mice	3h one session		–	–	[165]
Restraint stress	Male SD rats	6h per day, 14 days	↓	–	–	[166]
Restraint stress	Male Wistar rats	6h per day, 21 days	↓	↓	↓	[97,98]
Acute stress (cold immobilization, 1h and forced swimming 30 minutes)	Male Wistar rats	1h and 30 minutes	⇔	–	–	[106]
Maternal separation	SD rats	PND14-PND21	↓	–	–	[167,168]
Brief maternal separation	SD rats	PND 1-14,15 mins daily	⇔	⇔	⇔	[81]
Prolonged maternal separation	SD rats	PND 1-14, 180 mins daily	↓	⇔	⇔	[81]
Prenatal stress	SD rats	From day 15 until delivery restrained for 45 mins three times a day	↓	–	⇔	[105]
Unpredictable prenatal stress	Adult rats	Last week of pregnancy	↓	–	↓	[169]
Chronic unpredictable stress (CUS)	Male Wistar rats	21 days	↓	⇔	⇔	[170]
CUS	Male Wistar rats	21 days	↓	↓	–	[171]
Chronic mild stress	Swiss albino mice	5 weeks	↓	–	–	[172]
Chronic mild stress	SD rats	19 days	⇔	↓	⇔	[110]
Chronic mild stress	Male Wistar rats	14 days	↓	↓	↓	[103]
Chronic intermittent stress	Male SD rats	6h per day, 14 days	↓	–	–	[99]
Isolation stress	Tg2576 transgenic mice and non-transgenic littermates	From the time of weaning	↓	–	–	[173]

↓ decrease; ↑ increase; ⇔ no change, SD: Sprague Dawley, PND: Post-natal day

exogenous corticosterone suppresses neurogenesis both during the early post-natal period and in adulthood.[115,116] By contrast, adrenalectomy with low-dose corticosterone replacement in the drinking water, which prevents the stress-induced rise in corticosterone while maintaining its diurnal rhythm, eliminates the stress' effect on neurogenesis in the adult DG.[81,94] Collectively, these evidences suggest that stress inhibits cell proliferation and formation of new neurons by increasing glucocorticoids, at least in part.

Also, decreased neurogenesis in response to stress or corticosterone can be blocked by pre-treatment with an N-methyl-D-aspartate (NMDA) receptor antagonist, demonstrating a role for enhanced glutamate transmission.[117] Both, acute treatment with NMDA receptor antagonists and lesion of the entorhinal cortex independently increased the birth of cells in the DG.[118] An earlier study showed that the neuronal vulnerability to chronic restraint stress can be attenuated by entorhinal glutamatergic denervation.[83] Thus, the enhanced glutamate transmission following stress could also contribute to decreased hippocampal neurogenesis. Apart from these, stress is also reported to decrease levels of growth factors including brain-derived neurotrophic factor (BDNF), basic fibroblast growth factor (bFGF), insulin-like growth factor–1 (IGF-1), that are involved in the regulation of adult neurogenesis[119] and various neurochemical changes that could contribute to decreased neurogenesis.[120]

Numerous studies have linked adult neurogenesis with functions of the hippocampus, including learning and memory,[121] as well as with the development of

psychopathology and recovery from brain damage. The studies reviewed thus far present the possibility that stress-induced changes in neurogenesis may have cumulative effects that ultimately alter one or more of these brain processes.

NEUROTRANSMITTERS AND NEUROGENESIS

Amongst the myriad of extrinsic factors, many afferent inputs and various neurotransmitters, including classic (such as DA, serotonin, ACh, and glutamate), peptide (e.g., PACAP) and gaseous (e.g., nitric oxide) neurotransmitters have also been implicated in regulating adult neurogenesis.[23,30] The role of glutamate, GABA, serotonin and other neurotransmitters are reviewed elsewhere.[122-125] We discuss the specific role of ACh and DA in the regulation of adult neurogenesis.

Acetylcholine

The hippocampal formation receives abundant regulatory inputs from the basal forebrain cholinergic system, and ACh plays an important role both in learning and in the cognitive deficits associated with ageing and Alzheimer disease (AD).[126,127] Since the cholinergic system is closely associated with learning and memory and evidences have suggested a probable role for neurogenesis in learning, a few studies have investigated the role of ACh on the formation of new hippocampal neurons and its relation to learning and memory. Kaneko *et al*[128] showed using vChAT, a marker for the cholinergic nerve terminals that a high density of cholinergic innervation is present at the SGZ and the inner granule cell layer. Further, these cholinergic fibers made contact with the PSA-NCAM-positive neurons indicating that these new cells could potentially respond to cholinergic inputs. Neurotoxic lesion of forebrain cholinergic inputs with 192IgG Saporin was found to decrease proliferation, short-term survival,[129] and differentiation of the new-born cells into neurons[130] and increase apoptosis[130] in the hippocampus. This was associated with impaired performance in the water maze task.[129] Partial excitotoxic lesioning of the medial septum by infusion of NMDA significantly reduced survival of newly generated neurons by approximately 40% while the proliferation of progenitors remained unaffected.[131]

Kotani *et al*[132] showed that chronic treatment with donepezil but not scopolamine increased, the number of BrdU-positive cells in the DG compared to controls without affecting differentiation. Systemic administration of the cholinergic agonist physostigmine increased DG neurogenesis and the new cells were found to express muscarinic M1 and M4 receptors.[129] In an interesting study, it was shown that the muscarinic agonist, oxotremorine rescues the impaired neurogenesis induced by forebrain cholinergic lesions.[133] Homopentameric α7–containing and heteropentameric β2–containing nictonic ACh receptors (nAChR) are expressed by the new-born cells.[128,134] Chronic nicotine exposure results in decreased proliferation and β2 nAChR-knock-out mice display lesser proliferation, but normal survival.[135,136] Recently, it was shown that α7–nAChR are involved in the survival, and morpho-functional maturation of the new-born neurons.[137] These studies suggest that alterations in the cholinergic system plays an important role in the regulation of adult neurogenesis in the hippocampus.

Dopamine

DA is an important neurotransmitter implicated in the regulation of mood, motivation and movement.[137,138] In addition to being a neurotransmitter, it is also found to play a role in the regulation of endogenous neurogenesis in the adult mammalian brain. Early during embryonic development, DA regulates neural precursor cell proliferation[138] and its receptors appear in the highly proliferative germinal zones of the brain.[139,140]

DA has been shown to either activate or inhibit, proliferation of precursor cells in the lateral ganglionic eminence through D_1- and D_2-like receptors.[141,142] Immunohistochemical and electron microscopy studies in the SVZ have shown that D_2-like DAergic receptors are expressed predominately on C-cells (frequently dividing transit-amplifying cells), whereas A-cells (PSA-NCAM$^-$ positive-restricted neural precursors) express both D_1- and D_2-like receptors.[143] Furthermore, immunohistochemical studies with both confocal and electron microscopy[143] have demonstrated that C-cells in the adult SVZ are lodged in a rich network of dopaminergic afferents that form synapse-like structures.

Neurospheres have been demonstrated to express D_1- and D_2-like DAergic receptors.[143-145] Treatment of neurospheres with bromocriptine and apomorphine, significantly increased cell proliferation[143,144] which was blocked by the D_2-like antagonist sulpiride,[143,144] suggesting that it was indeed mediated via the D_2-like receptors. Neurotoxic lesion of the dopaminergic innervation to the forebrain in mice and rats using MPTP and 6-hydroxydopamine (6-OHDA) decreases global cell proliferation in the SVZ by 30–45%.[143,146,147] While high levels of D_3 receptor expression persists in the germinal SVZ,[139] 7-hydroxy-N,N-di-n-propyl-2-aminotetralin (7-OH-DPAT), a preferential D_3 receptor agonist does not alter neurogenesis in the SVZ of adult mice following intraventricular infusion.[148]

The inhibition of dopaminergic transmission in adult rats *in vivo* using the D_2-like antagonist haloperidol increased the proliferation of forebrain precursor cells on one hand, while did not alter the number of BrdU-labeled subependymal cells on the other.[145,149] In the same study, the authors also show that addition of DA or quinpirole (a selective $D_{2/3}$ receptor agonist), but not SKF 38393 (a selective D_1 receptor agonist), to neurosphere cultures derived from wild-type mice produced a dose-dependent (and approaching complete) inhibition of neurosphere formation.[145] D_2-like agonists like ropinirole or 7-OH-DPAT and levodopa increases precursor cell proliferation in the SVZ in both control and lesioned animals.[143,150]

The presence of DA transporter (DAT)–positive DAergic fibers was reported to be present near a BrdU–positive cell in the SGZ.[143] It was also reported that following MPTP lesion of the nigrostriatal pathway, there is decreased proliferation until 7 days following the lesion.[143] After the administration of haloperidol, no effect,[151,152] decrease,[149] or increase[153] in the proliferation in the SGZ have all been reported. Further, survival and differentiation in the DG is either not affected[152,154] or increased[155] following haloperidol administration. Activation of D_2 receptors by quinpirole increased the cell proliferation in the SGZ[156] and *in vitro* and *in vivo* experiments show that the effects of quinpirole are mediated by the ciliary neurotrophic factor (CNTF).[156] In animal models of schizophrenia, one study reported that haloperidol restores[153] proliferation while the other did not.[157] Proliferation of the precursor cells in the SGZ decreased following unilateral lesion of substantia nigra pars compacta (SNpc) by 6-OHDA and was restored with subchronic fluoxetine, a selective serotonin reuptake inhibitor but not with maprotiline, a selective norepinephrine reuptake inhibitor.[158] MPTP-lesioned mice had lesser number of PCNA–positive cells indicating decreased precursor cell proliferation in the SGZ.[143] Contrastingly in another study, there was a transient increase in cell proliferation 14 days post-ablation specifically with the amplifying neural progenitor cells and post-mitotic progeny, with no such effect seen after 4 days post-ablation.[159] However, this increase was restored to normal by 30 days of post-ablation. L-DOPA per se did not affect progenitor cell division in the DG and its administration immediately following MPTP treatment did not alter the effect of MPTP. Further, lesion alone did not significantly change the maturation of the precursors, but L-DOPA administration following the lesion enhanced the maturation.[159] Although this suggests a complex regulatory role of DA on neurogenesis, general compensatory mechanisms could also be responsible for the transient activation of neurogenesis following the lesion. Definitely more studies are required to clearly understand the role of DA on neurogenesis in the SGZ.

Evidence for reduced neurogenesis in the SVZ and SGZ in the postmortem brains of Parkinson disease (PD) patients[143] might underlie some of the non-motor cognitive symptoms in PD. A large body of converging data consistently shows that DA stimulates endogenous adult neurogenesis in the SVZ by activating D_2-like receptors on transit-amplifying progenitor cells. Further, as precursor cells in the adult mammalian brain are pharmacologically accessible to the systemic administration of dopamimetic drugs, the stimulation of endogenous neurogenesis appears to be a potential strategy for a cell replacement therapy of the brain in diseases in which progenitor cells appear to contribute to repair processes.[160-163]

PERSPECTIVES

Although considerable research has been carried out on adult neurogenesis in the past few years, we are only beginning to have a clear understanding as to how adult neurogenesis is regulated in health and disease, and what its functional implications are. With novel genetic tools available to study the properties of the new-born neurons, we could hope for a clearer picture of the players involved in the regulation of adult neurogenesis in the future. Insight into the basic process and molecular mechanisms of adult neurogenesis not only enriches our knowledge about the unique plastic properties of the adult brain, but also could significantly impact cell replacement therapies for psychiatric and degenerative neurological disorders.

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