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CHAPTER 9

ROLE OF HIPPOCAMPAL NEUROGENESIS IN THE PATHOPHYSIOLOGY AND TREATMENT OF DEPRESSION

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Abstract: Depression and anxiety are psychiatric illnesses that are major burdens on society and affect as much as 7% of the world's population. Here we review common approaches used to model depression in rodents such as chronic mild stress (CMS), social defeat, and chronic corticosterone treatment. We discuss the pros and cons of these different approaches. Furthermore, we provide a detailed review of adult hippocampal neurogenesis, including the distinct phases that a cell passes through when transitioning from precursor to neuron. Finally, we discuss at length the experiments that have related adult hippocampal neurogenesis to treatments of depression and anxiety, and why neurogenesis might be necessary for these treatments. We end by revisiting the neurogenesis hypothesis of depression and by providing suggestions for future research directions.

Keywords: Subventricular (SVZ) and subgranular zone (SGZ) of dentate gyrus (DG) of hippocampus, doublecortin (DCX) expressing cells, adult neurogenesis, cell differentiation, depression, antidepressant drugs, animal models.

1. INTRODUCTION

Depressive and anxiety disorders are a major burden on society. Mood disorders affect 7% of the world's population, while severe forms of depression impact 2-5% of the US population [1, 2]. Furthermore, approximately 32-35 million adults in the US population [16%] experience an episode of major depression in their lifetime [3]. The heterogeneous nature of depression suggests an involvement of multiple distinct brain regions, which may be responsible for the diverse symptoms. Human imaging and post-mortem studies have supported this hypothesis, implicating brain areas including the prefrontal and cingulate cortex, hippocampus, striatum, amygdala, and thalamus [4]. Together, these brain regions operate a series of highly interacting circuits that forms a neural circuitry involved in depression [5]**.** The hippocampus is one of several limbic structures that have been extensively studied in individuals with psychiatric and neurologic disorders in the last decade [5, 6]. Besides its critical role in learning and memory, the hippocampus is one of only two areas in mammalian brain where adult neurogenesis occurs [6]. Adult hippocampal neurogenesis is therefore defined as the progression from neural stem cell to mature dentate granule neuron.

Moreover, while many classes of drugs with antidepressant activity have been developed and approved [7], many patients do not respond to treatment [8]. Therefore it is critical for basic research to develop animal models that present behavioral, neurochemical and brain morphological phenotypes reminiscent of depression and anxiety. Given that anxiety and depression have a high comorbidity

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with co-occurrence rates up to 60% in patients [9, 10], animal models that present signs of both diseases could potentially be the most useful.

To fully understand the pathophysiology and treatment of depression, it is essential to delineate molecular, cellular and circuit-level determinants of chronic antidepressant action in addition to behavioral models. Of the current leading hypotheses of the pathophysiology and treatment of depression, the Neurogenesis Hypothesis of Depression deserves particular attention because it allows the characterization because changes in neurogenesis are only seen after chronic, but not acute, antidepressant treatment. This review revisits the role of adult hippocampal neurogenesis in the pathophysiology of mood disorders, especially anxiety/depression, and also in the antidepressant responses, especially in non-stressed and stressed rodents.

2. HIPPOCAMPAL NEUROGENESIS

Neurogenesis refers to the production of new neurons in the brain. Originally, it was only described during development. The dogma of Ramon y Cajal (1913) that the adult brain was unable of generating new neurons was first questioned by the work of Altman in the 1960s, who revealed the genesis of new cells in the brain of adult rat and cat by autoradiography with tritiated thymidine [11]. Unfortunately, it was uncertain whether the new cells were actually neuronal cells. The work of Kaplan and Hinds (1977) [12] confirmed this by using an ultrastructural analysis of the labeled cells. Many years later, a combination of specific neuronal markers and an analogue of thyminidine, 5-bromo-2'-deoxy-uridine (BrdU), confirmed the neuronal phenotype [13]. The process of adult neurogenesis is located in two discrete brain regions: the subventricular zone (SVZ) and subgranular zone (SGZ) of dentate gyrus of the hippocampus. In this chapter, only the hippocampal neurogenesis and its involvement in depression will be presented.

2.1. PRODUCTION OF NEW NEURONS IN THE SGZ

Hippocampal neurogenesis is possible in the SGZ of the dentate gyrus of the hippocampus because of to the presence of stem cells. These stem cells evolve into neural progenitor cells that can produce multiple cell types in the central nervous system such as neurons, astrocytes, oligodendrocytes, or microglial cells [14]. In rodents, the duration of the mitotic cycle of proliferating precursors is approximately 12 to 24 hours, leading to the production of about 8,000 to 10,000 new neurons per day [15] Given that the dentate gyrus consists of approximately one million granule cells, this phenomenon is capable of generating a little less than 1% of total granule cells each day. However, the proportion of new neurons that survive beyond one month is less than 50%, and the production of new cells is offset by the daily loss of mature granule cells. The surviving cells are predominantly a neuronal phenotype (75%), mainly glutamatergic granule cells, but also some are GABAergic interneuron basket cells. A smaller proportion of cells differentiate into astrocytes (15%), oligodendrocytes or microglia [16].

2.2. NEUROGENIC NICHE

Adult hippocampal neurogenesis, therefore, mainly refers to the production of a single neuronal type: the granule cells of the dentate gyrus. These cells are the principal excitatory neurons of the dentate gyrus (DG), receiving connections from the entorhinal cortex and sending projections along mossy fibers into the CA3 subfield of the hippocampus, where they terminate in structures rich in synapses and interneurons. Several projections are also sent into the hilus. The precursors are located between the hilus and the granular zone, in a niche called the subgranular zone (SGZ). The SGZ provides a

microenvironment that promotes neuronal development. This neurogenic niche includes precursors, immature neurons, glial and endothelial cells, microglia and some immune cells. The niche is surrounded by a basement membrane. Because of the important role of vascularization in this region, this niche is also called a vascular niche [17]. In all cases, the niche provides a unique environment for neuronal development. Studies suggest that astrocytes have a local key role in neurogenesis. *In vivo*, the development of cells has a preferred spatial relationship with astrocytes. *Ex vivo*, astrocytes and astrocyte-derived factors are potent inducers of hippocampal neurogenesis for the precursors [18]. The SGZ is also special because it receives connections from several brain regions: dopamine fibers in the ventral tegmental area, projections of serotonergic raphe nuclei, septum acetylcholine connections, and connections of GABAergic local interneurons. Manipulations of these neurotransmitter systems has also demonstrated regulatory effects on neurogenesis [19] [20]. The fact that these neurotransmitter systems regulate neurogenesis is of particular interest because antidepressants mainly act by changing monoamine levels [see later section entitled ANTIDEPRESSANTS AND ADULT HIPPOCAMPAL NEUROGENESIS]. A prominent example is that mice deficient in the Serotonin 1A receptor do not show a neurogenic response to chronic fluoxetine treatment [21].

2.3. CELLULAR STAGES OF HIPPOCAMPAL NEUROGENESIS

Neurogenesis can be divided into four phases: a step in which precursor cells are dividing, then surviving, a post mitotic maturation phase, and a late phase of survival.

2.3.1. The stage of precursor cell

The precursors have properties reminiscent of glial cells, including the morphology of radial glia. The cell body is located in the subgranular zone and the dendrites extend into the molecular layer. The nature of hippocampal astrocyte precursors was first revealed by [22]. Type 1 precursor cells yield intermediate progenitor cells, or type-2 cells, with a high proliferation activity. A subset of these cells continue to express glial markers, but lack the morphological characteristics of radial cells (type-2a). The type-2 cells that express nestin filaments (such as cell type-1) also express markers such as NEUROD1 and Prox1. This particular phenotype is used to classify cells as type-2b [16, 23]. Prox1 is specific to the development of granule cells. In type 2 cells, the developing cells receive GABAergic innervation [24], and are more sensitive to GABAergic stimulation. Similarly, type-1 cells respond to these stimuli by increasing cell proliferation [25]. Among the early expressed neuronal markers, doublecortin (DCX) is expressed on the type-2b cells. The expression of DCX extends from the proliferation phase to post mitotic maturation, which lasts about 2-3 weeks [26].

2.3.2. The phase of survival

Soon after the release of the cell cycle, new neurons express markers, such NeuN ["Specific neuronal nuclear protein"] and the transient marker calretinin [27]. A majority of cells fail to establish connections with the molecular layer and die. In contrast, cells that establish stable connections develop a stable dendritic arborization that extends in a specific way in time. In the days following the exit of the cell cycle, new cells emanate their axons to their target region CA3 to form appropriate synapses. This period is marked by the expression of a protein mediator, such as TOAD-64 or TUC-4, implicated in axon guidance. The first GABAergic innervations are excitatory and then become inhibitory when glutamatergic connections are established [24]. The GABAergic action leads cells to mature into glutamatergic cells and to develop synaptic integration [28] Most regulatory elements and processes occur at the stage of neuronal development, and rarely during the expansion phase [27, 29].

2.3.3. The maturation step; from early to late stage

Doublecortin is expressed in granule cells that range from 1 day to about 4 weeks of age, with 20% of its total population being proliferating neuroblasts and the rest being post-mitotic neurons [30].

Another marker of maturation, called calretinin, is also used as a marker for immature GCs at the early postmitotic stage. The period of expression of calretinin (3-4 weeks), is closely linked to dendritic maturation. However, using a lentiviral approach expressing the Green Fluorescent protein, it has been shown that the axonal growth to CA3 precedes the development of dendritic arborization [14]. After full integration to existing circuits, the new cells move from the calcium-binding protein calretinin to calbindin [27]. It is several weeks before the cells are no longer distinguishable electrophysiologically from mature neurons of the granule cells [31]. It has been postulated that the time needed to complete the functionality of these young neurons is related to the formation and storage of new elements of memory [32]. Finally, calbindin-like immunoreactivity has been described as a marker for mature granule cells.

2.4. QUANTITATIVE ANALYSIS OF PROLIFERATION, SURVIVAL, MATURATION AND DIFFERENTIATION, NEWBORN CELLS.

For a full characterization of the neurogenic effects of new compounds, all the steps of neurogenesis, including proliferation, survival, maturation, and differentiation have to be completed. So far, no specific and exclusive marker has been identified that would allow for prospective studies of neurogenesis. As a result, detection of neurogenic steps depends on a combination of labeling approaches. Thus, proliferation, differentiation and survival steps each require a specific protocol using the administration of a synthetic thymidine analogue, "5-bromo-3'-deoxyuridine" (BrdU) that substitutes for thymidine incorporation into DNA synthesized during the S phase of the cycle. Quantitative analysis of proliferation, differentiation, and survival of newborn cells is made by varying the time interval between the pulse administration of BrdU and the sacrifice of animals [33].

2.4.1. Main methods for a Detection of cell proliferation

For the quantification of rate of cell division, animals are administered BrdU [between 50 to 150 mg/kg 2h before sacrifice [34]. Proliferation is quantified by counting BrdU-positive cells. To simplify the experiment, quantification of BrdU-positive clusters can also be performed to measure proliferation since a positive correlation exists between BrdU-positive clusters and BrdU-positive cells. Since the quantification of BrdU-positive clusters is much less time consuming than counting BrdU-positive cells, this method can be used as a rapid indicator of the neurogenic effect of drugs or other manipulations [33]. This is important also because BrdU immunostaining has been used not only to test whether new drugs affect adult hippocampal neurogenesis, but also whether the anxiety/depressive-like state has been related to changes in hippocampal neurogenesis.

2.4.2. Endogenous markers of cell proliferation

Endogenous cell cycle proteins are expressed at different stages of cell cycle progression. For exemple the "Proliferating Cell Nuclear Antigen, PCNA" is expressed throughout the cell cycle and provide low temporal resolution. Ki-67 closely corresponds to BrdU as it is expressed both during S phase and through the remainder of the cell cycle (Figure **1**).

2.4.3. Survival and fate of the newly generated cells

Usually, for the quantification of rate of cell survival, animals are administered BrdU (100 to 150 mg/kg) twice a day during three days, 3 to 4 weeks before sacrifice [35]. The reason to wait for sacrifice is that the fate of the newly generated cells can only be determined three to four weeks later, once neuronal migration has occurred [13, 36]. Once progenitor cells reach maturity they begin expressing neuronal markers such as neurofilaments, NeuN, and Calcium-binding proteins or astrocyte markers such as glial fibrillary acidic protein (GFAP). Calcium-binding proteins such as calbindin are produced when cells become electrophysiological active. It is not until 4 weeks after birth that newly generated granule cells have acquired the typical features of mature granule cells and cease to express immature neuronal markers.

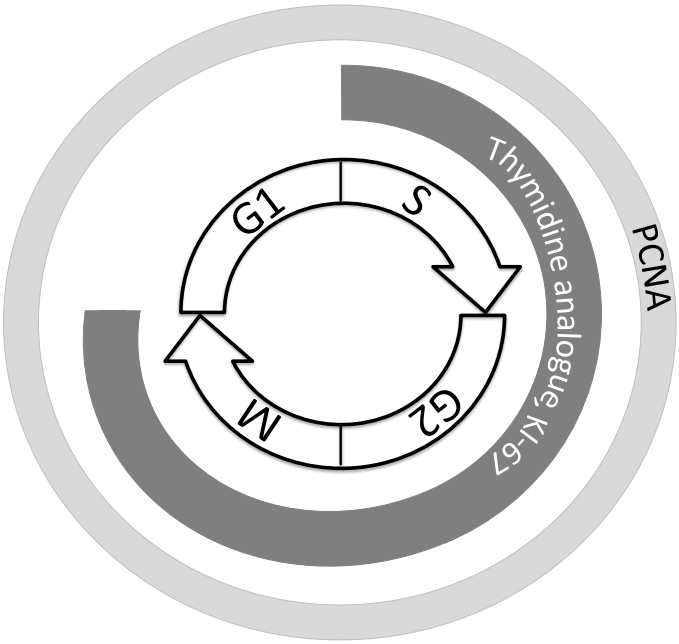

Figure 1: Schema representing the endogenous or exogenous markers used to study adult neurogenesis

2.4.4. Markers of maturation

Several markers have been used to assess maturation such as microtubule-associated protein [doublecortin]. In the adult dentate gyrus, DCX is exclusively expressed in immature neurons [26, 37], and thus has been widely used as an immature neuronal marker that reliably reflects the level of neurogenesis and its modulation [26]. To assess the impact of antidepressants on dendritic maturation, the morphology of cells that express doublecortin (DCX) are examined. DCX-positive cells with tertiary dendrites, which display more complex dendritic arborization, can be distinguished from others DCX cells. The ratio of DCX-positive cells with tertiary dendrites over total DCX-positive cells is informative of the rate of maturation. The polysialylated form of the neural cell adhesion molecule (PSA-NCAM) also has a transient expression pattern that marks neuronal progenitors. However, it should be noted that, in contrary to DCX, PSA-NCAM has also been detected in glial cells.

2.5. METHODS USED TO ABLATE OR INCREASE NEUROGENESIS

The analysis of the causal relationship between neurogenesis and behavior came from the removal of progenitor cells. Several methods to date have been developed to reduce or abolish neurogenesis (Table **1**):

An X-irradiation of either the whole brain or locally in the hippocampus [21];

A systemic treatment with an anti-mitotic agent such as methylazoxymethanol acetate [MAM] [38];

By genetic manipulation, such as GFAP-TK mice, in which the GFAP + progenitor cells die following treatment with ganciclovir [39].

In a contrary experiment, Sahay and colleagues [40] developed a genetic gain-of-function strategy to inducibly augment the survival of adult-born neurons in a cell-autonomous manner [40]. Because

Pharmacological manipulation	Cranial Irradiation	Genetic manipulation
5-fluorouracil [5-Fu], methylazoxymethanol [MAM], temozolomide [TMZ] cytosine arabinoside [Ara-C]	X-irradiation, gamma-rays	GFAP-TK, Nestin-TK, Nestin-Bax, $TrkB^{lox/lox\text{-}Cre\ ERT2}$ Cyclin D2 transgenic
[38] [96] [115]	[21, 39, 52, 69, 73, 97, 116-118]	[6, 51, 100, 101, 114, 119];

Table 1: Methods used to ablate or increase neurogenesis

60–80% of young adult-born neurons undergo programmed cell death, for which the pro-apoptotic gene Bax is required, they used a transgenic mouse line in which the tamoxifen (TAM)-regulatable recombinase CreERT2 is expressed under the control of a 5.26-kilobase fragment of the rat nestin (Nes) gene promoter [41] together with a floxed Bax mouse line to ablate Bax selectively in neural stem cells in the adult brain and promote survival.

3. HIPPOCAMPAL NEUROGENESIS AND MAJOR DEPRESSION AND ITS TREATMENT

3.1. THE ANXIETY / DEPRESSION STATE AND HIPPOCAMPAL NEUROGENESIS

The neurogenic hypothesis of depression postulates that decreased production of new granule cells in the dentate gyrus of the hippocampus is linked to the pathophysiology of depression and that the increase in hippocampal neurogenesis is required for the behavioral effects of antidepressant treatment [42]. The few studies of hippocampal neurogenesis in depressed patients published to date have mainly relied on histological examinations of post-mortem brain tissue and studies of magnetic resonance imaging. Thus, a reduction in hippocampal volume in depressed patients is somewhat established, and the contribution of two meta-analysis confirmed this reduction in hippocampal volume in patients with depression [43]. The frequency of depressive episodes and the period during which they are not treated coincide with the severity of the decline in hippocampal volume. However, pathophysiological studies on post-mortem brain tissue indicates that the change in the number of neuropil and glial cells may be responsible for reducing the volume of the hippocampus [44]. It is highly unlikely that loss of hippocampal neurogenesis can account for the decreased volume. These imaging results, involving hippocampal neurogenesis in the pathophysiology of major depressive episodes, have been challenged by a recent histology study [45]. This study did not actually detect the difference in stem cell proliferation [by Ki-67] in the hippocampus between patients with depression and healthy volunteers. The results of this study are limited, however, since the patients were on antidepressant medication at the time of death, which could mask the specific effects of depression on cell proliferation. In addition, in the absence of toxicology studies, it is not clear that the patients observed treatment. Moreover, given the diversity in the stages of neurogenesis, it is difficult to conclude any involvement of neurogenesis after only observation of cell proliferation. In contrast, a more recent study actually showed a 50% decrease in proliferation [by Ki-67] in patients with depression than in controls [46, 47]. However, this difference did not reach statistical significance.

Preclinical studies are useful to prove causal links between hippocampal neurogenesis and behavior. Using exposure to different types of stress, like chronic stress or social submission, causes a

decrease in cell proliferation in the hippocampus. It is important to keep in mind the disadvantages of these methods, such as non-specific effects of ablation that can involve structures other than the hippocampus, and therefore other functions. Dysfunction of hippocampal neurogenesis is only hypothesized to be part of the pathogenesis of major depressive disorders [48], [23]. In reality, the suppression of hippocampal neurogenesis in mice does not alter anxiety behavior in the open field or the light/dark paradigms, the elevated plus maze, or novelty suppressed feeding [49], [21], [39]. Thus, the X-irradiation in the hippocampus has no effect in the previously mentioned paradigms, suggesting that the loss of hippocampal neurogenesis is not sufficient for a behavioral phenotype of anxiety/depression, and does not exacerbate those induced by stress. Similarly, ablation of neurogenesis by MAM, a pharmacological agent, is not sufficient to induce an anhedonic behavior in rats [38]. Airan *et al* [50] explored in more detail the link between depression and hippocampal neurogenesis. It is clear from their study that chronic stress in rats is not associated with a decrease in neurogenesis, and that the removal of neurogenesis does not induce a depressive-like behavior [50]. However, other studies suggest that the mechanisms are more complex. Recently it was shown that adult hippocampal neurogenesis plays an important role in the regulation of affective states [51]. Indeed, transgenic mice that overexpress the protein Bax in apoptotic progenitor cells and thus have a deficit in neurogenesis, have an anxiety phenotype. In sum, there is evidence indicating that neurogenesis is not a major factor in the development of depression, but may be necessary for the behavioral effects of antidepressants [2].

4. ANTIDEPRESSANTS AND ADULT HIPPOCAMPAL NEUROGENESIS

4.1. IN PHYSIOLOGICAL CONDITIONS.

To study the effects of antidepressants in unstressed animals, the choice of the strain is essential. It should be noted that 129SvEv mice express a low basal level of proliferating cells in the SGZ, and therefore are more relevant to study an induced increase in antidepressant treatment in conditions without stress than BALB / cJ or C57Bl / 6 mice, which express a higher cell proliferation [52]. Instead, the study of the impact of stress on hippocampal neurogenesis is more relevant in these last two strains.

Effects of antidepressants on the proliferation and cell survival. In addition to the effects of fluoxetine [18 mg/kg] in increasing cell proliferation in 129/SvEvTAc mice [21] [Table **2**], it also increases the survival of postmitotic granule cells. These effects of SSRIs on proliferation and cell survival were also found in rats [53, 54]. It is important to note that the neurogenic effects of antidepressants are only seen with chronic treatment [21, 49, 54-56]. In addition, other treatments such as the atypical antidepressant tianeptine, electroconvulsive therapy, mood stabilizers such as lithium, and new antidepressants such as agomelatine, increase proliferation and cell survival in the adult hippocampus [57]. Furthermore, agomelatine selectively alters neurogenesis in the ventral hippocampus, a region more involved in the emotional response [58]. Therefore, it seems that proliferation and survival are regulated by distinct mechanisms. For example, an enriched environment increases the survival of immature cells without affecting proliferation [59]. In contrast, voluntary exercise increases the proliferation and survival, without affecting the maturation [30] or dendritic morphology of newborn neurons [60]. Finally, a recent study showed that fluoxetine targets a class of neural progenitor cells by directly increasing symmetric divisions [53]. Effects of antidepressants on the maturation. Until recently, it was not clear that SSRIs also targeted immature neurons by affecting their maturation and functional integration to the network. Wang and colleagues [2008] demonstrated that chronic treatment with fluoxetine stimulates maturation of young neurons [56]. Indeed, they have a dendritic

Table 2: Effects of chronic antidepressant treatment on hippocampal neurogenesis in rodents

Antidepressant	Specie	Dose (mg/kg)	Length of Treatment	Prolif.	Diff.	Survival	References
			25 days	=	ø	=	(120)
			21 days	↑	ø	ø	(121)
			7 days	=	ø	ø	
	Sprague Dawley Rat	5	1 day	=	ø	ø	(54)
			5 days	=	ø	ø	
			14 days	↑	ø	ø	
			28 days	↑	ø	ø	
		0.6	28 days	↑	ø	ø	(122)
	Wistar Rat	10	28 days	↑	↑	ø	(123)
	Lister Rat	7	14 days	↑	↑	ø	(124)
	(BALB/c * DBA/2) mice	10	21 days	↑	ø	ø	(125)
			10 days	↑	ø	ø	
			5 days	=	ø	ø	
Fluoxetine		?	?	↑			(126)
			5 days	=	ø	ø	
	S129 SvEv mice	10	11 days	↑	ø	ø	(21)
			28 days	↑	ø	↑	
		18	5 days	=	=	=	(56)
			28 days	↑	↑	↑	
	129Sl mice	18	21 days	↑	ø	ø	(127)
	A/J mice	18	21 days	=	ø	ø	
		18	28 days	=	=	ø	(52)
	BALB/cJ mice	10	28 days	=	=	=	(68)
		16	24 days	=	=	=	(128)
	C57BL/6 mice	16	24 days	=	=	=	
	CD1 mice	10	28 days	↑	=	↑	(129)
	DBA/2 mice	18	21 days	↑	ø	ø	(127)
	SWR/J mice	18	21 days	=	ø	ø	
			21 days	↑	↓	↑	
Agomelatine	Wistar Rat	40	7 days	=	ø	ø	(57)
			1 day	=	ø	ø	
AMPA	Rodents	?	?	↑	ø	ø	(130)
Glutamatergic antagonist	Rodents	?	?	↑	ø	ø	(131)
Interleukine beta 1receptor antagonist	Rodents	?	?	↑	ø	ø	(132)
Citalopram	Wistar Rat	10	28 days	=	=	=	(133)
ECT	Wistar Rat	1 fois par jour	5 days	↑	↑	ø	(134)

Legend : ↑, increase; ↓, decrease; =, no change; ø, not study

Antidepressant	Species	Dose (mg/kg)	Length of Treatment	Prolif.	Diff.	Survival	References
Fluoxéeine/adolescent	BALB/cJ mice	16	24 days	↑	=	=	(128)
	C57BL/6 mice	16	24 days	↑	↑	↑	
Imipramine	Wistar Rat	5	56 days	↑	ø	ø	(135)
	(BALB/c * DBA/2) mice	30	21 days	↑	ø	↑	(125)
	129 SvEv mice	20	28 days	↑	ø	↑	(21)
Lithium	Wistar Rat	2,5	14 days	↑	↑	↑	(136)
	C57BL/6 mice	2.4 g/kg	28 days	↑	=	↑	(137)
Olanzapine	Sprague Dawley Rat	2	21 days	↑	ø	ø	(121)
		2	7 days	=	ø	ø	
Reboxétine	Rat Sprague Dawley	20 (2/day)	21 days	↑	ø	ø	(54)
Riluzole	Rodents	?	?	↑	ø	ø	(138)
Rolipram	129 SvEv mice	?	?	↑		ø	(139)
Tesofensine	Wistar Rat	3	5 days	=	ø	=	(140)
		3	14 days	↑	ø	↑	
Tianeptine	Tree Shrews	50	28 days	=	ø	ø	(141)
Tranylcypromine	Sprague Dawley Rat	7.5, then 10 (14 last days)	21 days	↑	ø	ø	(142)
Venlafaxine	Wistar Rat	10	14 days	=	ø	ø	(143)
		40		↑	ø	ø	

Legend : ↑, increase; ↓, decrease; =, no change; ø, not study

arborization more complex than those present in untreated animals, suggesting an accelerating effect of fluoxetine on hippocampal neuronal maturation [56]. The duration of onset of fluoxetine effects on maturation also coincides with the time required for the onset of behavioral effects of this treatment in mice. Similarly, electroconvulsive therapy (ECT), which provides a very rapid therapeutic effect, stimulates neurogenesis faster than fluoxetine [61]. In addition, ECT stimulates dendritic development and maturation [62]. After ECT, young neurons express an increase in dendritic growth, and begin to receive glutamatergic synaptic connections early. These results suggest that molecules that increase the maturation of new neurons are targets for the development of future treatments.

4.1. EFFECTS OF ANTIDEPRESSANTS ON THE DIFFERENTIATION

Four weeks after birth, progenitor cells acquire properties of mature granule cells. For example, the new cells no longer express the immature neuronal markers DCX or PSA-NCAM, and receive the same GABAergic and glutamatergic afferents as mature granule cells of the dentate gyrus [14, 63, 64]. However, these new cells continue to mature morphologically and physiologically. The dendritic spines of a neuron at four weeks old are more associated with multiple synaptic boutons that older neurons and the density continues to increase even after 8 weeks [63]. In addition, neurons aged 2 to 4 weeks exhibit an increase in excitability and a lower threshold for induction of long-term potentiation (LTP), while at 4 to 6 weeks, new neurons exhibit greater LTP amplitude [65]. In addition, a specific form of LTP (ACSF-LTP) requires the hippocampal neurogenesis, as this form of LTP is blocked after ablation of neurogenesis [39]. This critical period in young neurons coincides with the development of receptor expression of NMDA NR2B [28].

At any given time following differentiation, young neurons can be found across stages of development and with distinct morphological and physiological characteristics. Similar to new neurons in the developing brain, the newborn granule cells depolarize in response to GABA because of their high intracellular concentration of chloride ions [66]. Somewhere between 2 to 4 weeks after birth the response to GABA changes from depolarization to hyperpolarization as the young cells develop their dendritic spines and receive glutamatergic connections. In addition, new granule cells begin to make connections with the hilus and CA3 region while synaptic complexity is similar to that of mature neurons [67].

4.2. DEPENDENT AND INDEPENDENT NEUROGENIC EFFECTS IN THE ACTION OF ANTIDEPRESSANTS

Recently Boldrini and colleagues [46] suggested that future studies in humans should determine at which level antidepressants should affect neurogenesis to maximize the response [46]. Preclinical studies in rodents, using approaches that override functional neurogenesis, are used to establish the relationship between neuronal activity and the contribution of hippocampal neurogenesis in the behavioral effects of antidepressants in animals that are either stressed or not. The study by Santarelli and colleagues [21] has raised many questions about the contribution of hippocampal neurogenesis in the behavioral improvement induced by antidepressants, unstressed mice [21]. From this study it was shown that the behavioral effect of SSRIs is dependent on the presence of hippocampal neurogenesis [56]. However, in BALB / cJ mice, abolishing neurogenesis by x-ray does not block the behavioral response induced by SSRIs in different behavioral paradigms related to anxiety / depression, especially the NSF [52]. Furthermore, chronic treatment with fluoxetine did not increase hippocampal neurogenesis [52, 68]. Likewise, the beneficial contributions of an enriched environment, physical exercise, or learning behavior related to anxiety appear to be independent of increased neurogenesis in adult mice [69].

To examine whether the increase induced by antidepressant treatment may simply be an epiphenomenon, the study of animal models of anxiety / depression in which hippocampal neurogenesis is reduced appears to be a good alternative.

5. ANIMAL MODELS OF DEPRESSIVE PHENOTYPES

Since no genetic variants with high penetrance that cause depression are known, animal models have mainly relied on different means of chronically exposing rodents to stressful experiences, or sensory tract lesions such as in olfactory bulbectomy, to induce behavioral states that present depression-like signs and are responsive to chronic antidepressant treatment.

The oldest most commonly used paradigm to induce a depression-like state is chronic mild stress (CMS). Initial observations suggested that animals subjected to multiple stressors over a prolonged period of time reduced their intake of saccharine or sucrose, a potential behavioral model of anhedonia [70]. Furthermore, this effect was selectively reversed by chronic treatment with the TCA imipramine [70]. Further work was able to repeat this result using more mild stressors, such as periods of food and water deprivation, small temperature reductions and changes of cage mates [71, 72]. Following these studies the CMS procedure, and modified versions such as chronic unpredictable stress (CUS or UCMS), became commonly used and much work demonstrated that other depression-like changes were induced in animals, such as decreased sexual and aggressive behavior, decreased self-care, and altered sleep patterns [71]. Furthermore these behaviors are all reversible by chronic, but not acute,

treatment using multiple classes of antidepressants [73]. While historically potential pitfalls of the CMS procedure are that it is notoriously labor intensive, and that there has been some difficulty in getting the procedure established and the results replicated across laboratories [74], the modified versions of the CMS have proven more useful.

Recently, there have been some reports using CMS or variants to model treatment resistance in rodents. In one study, CMS significantly decreased sucrose consumption and the proliferation of adult hippocampal neural progenitors [75]. Following chronic treatment with a SSRI (escitalopram), the subjects were retested for sucrose consumption. A bimodal distribution was found where one group recovered [increased sucrose consumption] while another refracted treatment [no increase in sucrose consumption]. Interestingly, there was a correlation between the animals in the group that recovered with a reversal of the decreased proliferation that was absent in the group resistant to treatment [75]. More recently, follow-up work has taken a proteomic approach in an attempt to find molecular differences in the ventral hippocampus between responders and non-responders [76]. Another study demonstrated that if animals are on a high fat diet during multiple UCMS procedures they become resistant to treatment with a SSRI (fluoxetine) [77].

A distinct procedure that has gained traction is the usage of a social defeat model. In this paradigm a mouse is forced into the territory of a mouse from a larger, more aggressive strain leading to an interaction resulting in intruder subordination. Repeated defeats over 10 days can result in a long lasting reduced social interaction, sexual dysfunction, sleep dysregulation, anxiety, metabolic deficits and anhedonia [78-81]. Interestingly, following the social defeat procedure there remains a large variance in behavior outcomes in spite of using an inbred mouse strain (C57BL/6). Some animals display a resistance to social defeat (resilience) while others are susceptible (determined by interaction with a social target relative to an empty enclosure). If animals are separated based on this measure, susceptible mice demonstrate decreased sucrose intake, a blunted circadian rhythm, and conditioned place preference to cocaine [80]. Furthermore, phenotypes induced by social defeat in susceptible mice can be reversed by antidepressant treatment [79]. Given that molecular mechanisms for resilience to the stressful procedure are now being worked out [80, 82], it would be intriguing to see if similar pathways are necessary for mediating response to antidepressants.

A third procedure for inducing a depression-like state in animals is administration of chronic glucocorticoids in order to mimic the effects of chronic stress. A significant proportion of depressed patients display altered activity of the HPA axis, and stress generally leads to hypersecretion of corticosteroids, which imposes an increased risk for depression [10, 37, 83-88]. Chronic treatment of rodents with corticosterone effectively induces multiple anxiety- and depression-like changes in behavior, neurochemistry and brain morphology [89-92]. Behaviorally, depression-related changes include suppression of sucrose intake and decreased self-care [90, 93], while anxiety-related changes include increased latency to emerge into the light compartment in the light/dark test, decreased time, entries and percent distance in the center of an open field and increased latency to take a bite of food in the novelty suppressed feeding (NSF) test [90, 91].

6. ADULT HIPPOCAMPAL NEUROGENESIS IN PATHOLOGICAL CONDITIONS

One of the main findings of the role of adult hippocampal neurogenesis in depression is the observation that antidepressants confer behavioral effects by stimulating neurogenesis in rodents and humans. A recent study showed for the first time in the dentate gyrus in humans that there are more nestin-expressing progenitor cells, and increased dividing cells after treatment with an SSRI antidepressant

(sertraline, fluoxetine) or tricyclic (nortriptyline, clomipramine) in patients with depression compared to untreated patients [46]. Another study also showed that antidepressants increase human hippocampal neurogenesis by activating the glucocortiocoid receptor [94]. In nonhuman primates, repeated separation stress resulted in depression-like behaviors [anhedonia and subordinance] accompanied by reduced hippocampal neurogenesis [95]. Treatment of the nonhuman primates with fluoxetine stimulated neurogenesis and prevented the emergence of depression-like behaviors. Furthermore, ablation of neurogenesis with irradiation of the nonhuman primates abolished the therapeutic effects of fluoxetine.

To address whether altered neurogenesis is important for the treatment of depression, Deisseroth's group used voltage sensitive dye imaging to probe hippocampal activity in the CMS in Rat and specifically the role of neurogenesis in depression-relevant neurophysiology and behavior [50]. Using irradiation to ablate neurogenesis, Airan and colleagues also found that antidepressant behavioral efficacy in the Forced Swim Test in Rat required intact neurogenesis. Overall, antidepressant treatment was sufficient to transiently increase neurogenesis and exert behavioral effects long after drug clearance from the system, and this effect was absent in animals lacking neurogenesis (X-Ray). Recently, an elegant study in Rat confirmed Deisseroth's study by showing that antidepressants retain some but not all their therapeutic efficacy in reducing measured indices of anxiety/depression-like behavior when hippocampal neurogenesis was blocked by a cytostatic agent [96]. Indeed, using CMS and the antimitotic agent MAM, authors showed that the various antidepressants ameliorated CMS-induced behavioral signs of depression to the same extent in vehicle and MAM-treated animals. Conversely, using the NSF paradigm, they found that the antidepressant drugs studied (imipramine, fluoxetine) reduced the hyperanxious state observed in CMS-exposed rats even though neurogeneis was blocked. Overall, authors concluded that antidepressants re-established neuronal plasticity in hippocampus. In the "CORT model", using X-irradiated mice, in which hippocampal neurogenesis was abolished, we demonstrated that antidepressant treatment still elicits some anxiolytic/antidepressant-like effects. Specifically we found that antidepressant effects in the Open Field and Forced Swim Test were neurogenesis independent, while effects in the Novelty Suppressed Feeding Test or on coat state were neurogenesis dependent. As such, our study reveals that the behavioral effects of fluoxetine are mediated through both neurogenesis-dependent and -independent actions [97]. Previously, Surget and colleagues [73] presented important evidence for both neurogenesis-dependent and -independent mechanisms for the reversal of stress-induced behaviors by antidepressant drugs, including fluoxetine [73]. Our paper, using a different model of stress, extends this study by utilizing a mechanistic approach to propose a neurogenesis-independent pathways for mediating the effects of SSRIs, namely the β-arrestin signaling pathway.

7. ADULT HIPPOCAMPAL NEUROGENESIS IN DIFFERENT GENDERS

Taking into consideration that depression is twice as common in women as in men, it is important to also consider sex differences in the effects of depression models and antidepressants on adult neurogenesis. In MRL/MpJ mice treated with fluoxetine (10 mg/kg b.i.d.) for 21 days, cell proliferation was increased in both genders, but females produced more new cells than males [95]. Furthermore, while fluoxetine did not alter survival in males, 10 mg/kg reduced survival in females. Another study showed that while acute stress reduced cell proliferation in males, it did not affect proliferation in the female hippocampus [98]. Repeated training with controllable stress did not influence proliferation in females and under all conditions, males were more likely than females to express helplessness behavior. This was true even for males that were not previously stressed. Another study also showed that female rats learn trace memories better than male rats and consequently retain a greater proportion

of new neurons in their hippocampi [99]. Therefore, it is critical to also consider gender when planning experiments to study adult neurogenesis.

8. POTENTIAL MECHANISMS UNDERLYING THE REQUIREMENT OF NEUROGENESIS IN MEDIATING THE ANTIDEPRESSANT RESPONSE

While much work has been done that has laid a foundation for the understanding of how antidepressants increase neurogenesis, much less is known about why the increase in neurogenesis is required for the antidepressant response [2]. One likely mechanism would be negative feedback regulation of the HPA axis and the stress response. Consistent with this hypothesis, recent studies demonstrated that in mice with ablation of neurogenesis there was an increased HPA axis response to an acute stress [100, 101]. Since stimulation of the subiculum, CA3 or DG can yield an inhibitory effect on the HPA axis [102, 103] through well-described circuitry [103-105], it is possible that young neurons may contribute to hippocampal-dependent negative feedback of the HPA axis. One recent study even suggests that chronic stress severely impairs HPA axis activity and the ability of the hippocampus to modulate downstream brain areas involved in the stress response [73]. Chronic antidepressant treatment can restore the relationship between the hippocampus and the HPA axis, but only in the presence of an intact neurogenic niche. Another study also demonstrates that adult-born hippocampal neurons are required for normal expression of the endocrine and behavioral components of the stress response [106]. Future studies will need to use genetic methods to more directly determine if young neurons impact the negative feedback circuit to the HPA axis.

Another hypothesis, which is not mutually exclusive, that has gained traction is whether neurogenesis in different areas of the SGZ play distinct roles in the regulation of mood. Due to participation in different circuitry, it has been suggested that the dorsal and ventral hippocampus may be distinct structures [58]. In the hippocampus, the dorsal dentate gyrus receives inputs from lateral and caudomedial entorhinal cortex and medially located cells of the medial septal nucleus [107]. Outputs of the dorsal hippocampus are to the mammillary complex, dorsal lateral septum and lateral entorhinal cortex. In contrast ventral dentate gyrus receives inputs from the rostromedial entorhinal cortex and laterally located cells of the medial septal nucleus while ventral hippocampus outputs are to the prefrontal cortex, amygdala, nucleus accumbens, hypothalamus, medial entorhinal cortex, bed nucleus of stria terminalis and rostral and ventral lateral septum [108]. This different circuitry may suggest that the dorsal hippocampus is more important for learning and memory while the ventral hippocampus is more involved in emotion [58, 108]. Some lesion studies have supported this hypothesis [109, 110]. Based on this work, it has been proposed that neurogenesis along the dorsal-ventral axis may also play distinct roles in learning and mood [108]. In this idea, the main effect of neurogenesis in the antidepressant response would be on circuitry through ventral structures. Genetic models and ablation techniques that are restricted to dorsal or ventral SGZ need to be developed in order to test this hypothesis.

Much work has been done to advance the understanding of the synaptic and physiological properties of the young neurons and these unique properties allow for distinguishing young neurons from their mature granule neuron counterparts [111, 112]. Of particular relevance to antidepressant treatment is a form of long-term potentiation derived from a weak stimulation paradigm in the absence of GABA blockers [ACSF-LTP] that is sensitive to manipulations that block hippocampal neurogenesis [39, 56]. After chronic, but not acute, fluoxetine treatment, ACSF-LTP is enhanced in sham animals and completely blocked in animals subjected to X-irradiation [56], suggesting an effect of fluoxetine on the electrophysiological properties of young neurons that have integrated into the hippocampal circuitry.

Relatively little work has addressed the function of young neurons in an intact hippocampal circuit *in vivo*. In hippocampal slice work, it has been demonstrated that fluoxetine treatment enhances activity of the dentate gyrus relative to CA1 in a neurogenesis-dependent manner [50], suggesting a network effect of the young neurons. Furthermore, one very recent study used multiple methods to ablate adult neurogenesis *in vivo* and assessed hippocampal activity [113]. In anesthetized mice after X-irradiation or thymidine kinase mediated pharmacogenetic ablation, perforant-path evoked responses were reduced in magnitude. In striking contrast, there was an increase in the amplitude of spontaneous gamma-frequency bursts in the dentate gyrus and hilus, as well as increased synchronization of dentate neuron firing to these bursts. This striking result may suggest that the young neurons can serve to modulate activity in the much larger population of mature granule cells rather than acting solely as independent encoding units [113]. One could imagine that antidepressant treatment may modulate hippocampal circuitry by enhancing this effect of the young neurons on the mature granule neurons, but this possibility remains to be tested.

9. REVISITING THE NEUROGENESIS HYPOTHESIS OF DEPRESSION

The neurogenesis hypothesis of depression postulated that a decrease in the production of newborn granule cells in the dentate gyrus is related to the pathophysiology of depression while enhanced hippocampal neurogenesis is required for the beneficial effects of antidepressant treatment. With few exceptions [51, 114], in most studies ablation of hippocampal neurogenesis alone is not sufficient to induce a phenotype reminiscent of either anxiety or depression [14, 21, 39, 50, 52, 69, 73, 107]. It is also unlikely that decreased neurogenesis could account for the volumetric decreases seen in the hippocampus of depressed patients, as X-irradiation of mouse hippocampus does not yield a significant reduction [21]. Whether specific manipulations that increase hippocampal neurogenesis alone result in a "non-depressed" phenotype remains to be tested. However, evidence is strong that neurogenesis is required for at least some of the beneficial effects of antidepressant treatment. It will be critical for future work to determine how the addition of new units to the dentate is involved in mediating the effects of antidepressants.

It will also be critical for future work to validate the importance of antidepressant-induced neurogenesis in translational studies in humans. It will be important to test if biomarkers (such as CBV and MRS) are increased in patients treated with antidepressants. Furthermore, it may be interesting to correlate rates of neurogenesis as measured by these biomarkers with improvement of depressive signs and symptoms.

REFERENCES

[1] Murray CJ, Lopez AD. Evidence-based health policy--lessons from the Global Burden of Disease Study. Science 1996, 274:740-3.

[2] Samuels BA, Hen R. Neurogenesis and affective disorders. Eur J Neurosci. 2011, 33:1152-9. Epub 2011/03/15.

[3] Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, *et al*. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA 2003, 289:3095-105.

[4] Drevets WC, Bogers W, Raichle ME. Functional anatomical correlates of antidepressant drug treatment assessed using PET measures of regional glucose metabolism. Eur Neuropsychopharmacol 2002, 12:527-44.

[5] Nestler EJ, Barrot M, DiLeone RJ, Eisch AJ, Gold SJ, Monteggia LM. Neurobiology of depression. Neuron 2002, 34:13-25.

[6] Eisch AJ, Cameron HA, Encinas JM, Meltzer LA, Ming GL, Overstreet-Wadiche LS. Adult neurogenesis, mental health, and mental illness: hope or hype? J Neurosci 2008, 28:11785-91.

[7] Wong EH, Tarazi FI, Shahid M. The effectiveness of multi-target agents in schizophrenia and mood disorders: Relevance of receptor signature to clinical action. Pharmacol Ther 2010, 126:173-85.

[8] Trivedi MH, Rush AJ, Wisniewski SR, Nierenberg AA, Warden D, Ritz L, *et al*. Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D: implications for clinical practice. Am J Psychiatry 2006, 163:28-40.

[9] Gorman JM. Comorbid depression and anxiety spectrum disorders. Depress Anxiety 1996, 4:160-8.

[10] Leonardo ED, Hen R. Genetics of affective and anxiety disorders. Annu Rev Psychol 2006, 57:117-37.

[11] Altman A, Gilmartin TD, Katz DH. Differentiation of murine bone marrow stem cells *in vitro*: long-term growth promoted by a lymphocyte-derived mediator. Science 1981, 211:65-7.

[12] Kaplan MS, Hinds JW. Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science 1977, 197:1092-4.

[13] Cameron HA, Gould E. Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. Neuroscience 1994, 6:203-9.

[14] Zhao S, Cunha C, Zhang F, Liu Q, Gloss B, Deisseroth K, *et al*. Improved expression of halorhodopsin for light-induced silencing of neuronal activity. Brain cell biology 2008, 36:141-54.

[15] McDonald HY, Wojtowicz JM. Dynamics of neurogenesis in the dentate gyrus of adult rats. Neurosci Lett 2005, 385: 70-5.

[16] Steiner B, Klempin F, Wang L, Kott M, Kettenmann H, Kempermann G. Type-2 cells as link between glial and neuronal lineage in adult hippocampal neurogenesis. Glia 2006, 54:805-14.

[17] Palmer TD, Willhoite AR, Gage FH. Vascular niche for adult hippocampal neurogenesis. The Journal of comparative neurology 2000, 425:479-94.

[18] Barkho BZ, Song H, Aimone JB, Smrt RD, Kuwabara T, Nakashima K, *et al*. Identification of astrocyte-expressed factors that modulate neural stem/progenitor cell differentiation. Stem Cells Dev 2006,15:407-21.

[19] Cooper-Kuhn CM, Winkler J, Kuhn HG. Decreased neurogenesis after cholinergic forebrain lesion in the adult rat. Journal of neuroscience research 2004, 77:155-65.

[20] Dominguez-Escriba L, Hernandez-Rabaza V, Soriano-Navarro M, Barcia JA, Romero FJ, Garcia-Verdugo JM, *et al*. Chronic cocaine exposure impairs progenitor proliferation but spares survival and maturation of neural precursors in adult rat dentate gyrus. The European journal of neuroscience 2006, 24:586-94.

[21] Santarelli L, Saxe M, Gross C, Surget A, Battaglia F, Dulawa S, *et al*. Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. Science 2003, 301:805-9.

[22] Seri B, Garcia-Verdugo JM, McEwen BS, Alvarez-Buylla A. Astrocytes give rise to new neurons in the adult mammalian hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 2001, 21:7153-60.

[23] Kempermann G, Kronenberg G. Depressed new neurons--adult hippocampal neurogenesis and a cellular plasticity hypothesis of major depression. Biological Psychiatry 2003, 54:499-503.

[24] Tozuka Y, Fukuda S, Namba T, Seki T, Hisatsune T. GABAergic excitation promotes neuronal differentiation in adult hippocampal progenitor cells. Neuron 2005, 47:803-15.

[25] Huttmann K, Sadgrove M, Wallraff A, Hinterkeuser S, Kirchhoff F, Steinhauser C, *et al*. Seizures preferentially stimulate proliferation of radial glia-like astrocytes in the adult dentate gyrus: functional and immunocytochemical analysis. The European journal of neuroscience 2003, 18:2769-78.

[26] Couillard-Despres S, Winner B, Schaubeck S, Aigner R, Vroemen M, Weidner N, *et al*. Doublecortin expression levels in adult brain reflect neurogenesis. The European journal of neuroscience 2005, 21:1-14.

[27] Brandt MD, Jessberger S, Steiner B, Kronenberg G, Reuter K, Bick-Sander A, *et al*. Transient calretinin expression defines early postmitotic step of neuronal differentiation in adult hippocampal neurogenesis of mice. Mol Cell Neurosci. 2003, 24:603-13.

[28] Ge S, Sailor KA, Ming GL, Song H. Synaptic integration and plasticity of new neurons in the adult hippocampus. J Physiol 2008, 586:3759-65.

[29] Kempermann G, Gast D, Kronenberg G, Yamaguchi M, Gage FH. Early determination and long-term persistence of adult-generated new neurons in the hippocampus of mice. Development 2003,130:391-9.

[30] Plumpe T, Ehninger D, Steiner B, Klempin F, Jessberger S, Brandt M, *et al*. Variability of doublecortin-associated dendrite maturation in adult hippocampal neurogenesis is independent of the regulation of precursor cell proliferation. BMC Neurosci 2006, 7:77.

[31] van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. Nature 2002, 415:1030-4.

[32] Aimone JB, Wiles J, Gage FH. Potential role for adult neurogenesis in the encoding of time in new memories. Nat Neurosci 2006, 9:723-7.

[33] David DJ, Wang J, Samuels BA, Rainer Q, David I, Gardier AM, *et al*. Implications of the functional integration of adult-born hippocampal neurons in anxiety-depression disorders. Neuroscientist 2010, 16:578-91.

[34] Taupin D. What value reassurance? J Gastroenterol Hepatol 2007, 22:2052-3.

[35] Taupin P. BrdU immunohistochemistry for studying adult neurogenesis: paradigms, pitfalls, limitations, and validation. Brain Res Rev 2007, 53:198-214.

[36] Paizanis E, Hamon M, Lanfumey L. Hippocampal neurogenesis, depressive disorders, and antidepressant therapy. Neural Plast 2007, 2007:73754.

[37] Brown ES, Varghese FP, McEwen BS. Association of depression with medical illness: does cortisol play a role? Biol Psychiatry 2004, 55:1-9.

[38] Jayatissa MN, Henningsen K, West MJ, Wiborg O. Decreased cell proliferation in the dentate gyrus does not associate with development of anhedonic-like symptoms in rats. Brain Res 2009, 1290:133-41.

[39] Saxe MD, Battaglia F, Wang JW, Malleret G, David DJ, Monckton JE, *et al*. Ablation of hippocampal neurogenesis impairs contextual fear conditioning and synaptic plasticity in the dentate gyrus. Proceedings of the National Academy of Sciences of the United States of America 2006,103:17501-6.

[40] Sahay A, Scobie KN, Hill AS, O'Carroll CM, Kheirbek MA, Burghardt NS, *et al*. Increasing adult hippocampal neurogenesis is sufficient to improve pattern separation. Nature 2011, 472:466-70.

[41] Dranovsky A, Picchini AM, Moadel T, Sisti AC, Yamada A, Kimura S, *et al*. Experience dictates stem cell fate in the adult hippocampus. Neuron 2011, 70:908-23.

[42] Sheline YI. Hippocampal atrophy in major depression: a result of depression-induced neurotoxicity? Mol Psychiatry 1996, 1:298-9.

[43] Videbech P, Ravnkilde B. Hippocampal volume and depression: a meta-analysis of MRI studies. The American journal of psychiatry 2004, 161:1957-66.

[44] Czeh B, Lucassen PJ. What causes the hippocampal volume decrease in depression? Are neurogenesis, glial changes and apoptosis implicated? Eur Arch Psychiatry Clin Neurosci 2007, 257:250-60.

[45] Reif A, Fritzen S, Finger M, Strobel A, Lauer M, Schmitt A, *et al*. Neural stem cell proliferation is decreased in schizophrenia, but not in depression. Mol Psychiatry 2006, 11:514-22.

[46] Boldrini M, Underwood MD, Hen R, Rosoklija GB, Dwork AJ, John Mann J, *et al*. Antidepressants increase neural progenitor cells in the human hippocampus. Neuropsychopharmacology. 2009, 34:2376-89.

[47] Boldrini M, Hen R, Underwood MD, Rosoklija GB, Dwork AJ, Mann JJ, *et al*. Hippocampal Angiogenesis and Progenitor Cell Proliferation Are Increased with Antidepressant Use in Major Depression. Biological Psychiatry 2012, 72:562-71.

[48] Duman RS, Malberg J, Nakagawa S, D'Sa C. Neuronal plasticity and survival in mood disorders. Biological Psychiatry 2000, 48:732-9.

[49] David DJ, Klemenhagen KC, Holick KA, Saxe MD, Mendez I, Santarelli L, *et al*. Efficacy of the MCHR1 antagonist N-[3-[1-{[4-[3,4-difluorophenoxy]phenyl]methyl}[4-piperidyl]]-4-methylphenyl]-2-methylpropanamide [SNAP 94847] in mouse models of anxiety and depression following acute and chronic administration is independent of hippocampal neurogenesis. J Pharmacol Exp Ther. 2007;321[1]:237-48.

[50] Airan RD, Meltzer LA, Roy M, Gong Y, Chen H, Deisseroth K. High-speed imaging reveals neurophysiological links to behavior in an animal model of depression. Science 2007, 317:819-23.

[51] Revest JM, Dupret D, Koehl M, Funk-Reiter C, Grosjean N, Piazza PV, *et al*. Adult hippocampal neurogenesis is involved in anxiety-related behaviors. Mol Psychiatry 2009, 14:959-67.

[52] Holick KA, Lee DC, Hen R, Dulawa SC. Behavioral effects of chronic fluoxetine in BALB/cJ mice do not require adult hippocampal neurogenesis or the serotonin 1A receptor. Neuropsychopharmacology 2008, 33:406-17.

[53] Encinas JM, Vaahtokari A, Enikolopov G. Fluoxetine targets early progenitor cells in the adult brain. Proc Natl Acad Sci U S A 2006, 103:8233-8.

[54] Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 2000, 20:9104-10.

[55] Duman RS, Malberg J, Nakagawa S. Regulation of adult neurogenesis by psychotropic drugs and stress. J Pharmacol Exp Ther 2001, 299:401-7.

[56] Wang P, Lushnikova T, Odvody J, Greiner TC, Jones SN, Eischen CM. Elevated Mdm2 expression induces chromosomal instability and confers a survival and growth advantage to B cells. Oncogene 2008, 27:1590-8.

[57] Banasr M, Soumier A, Hery M, Mocaer E, Daszuta A. Agomelatine, a new antidepressant, induces regional changes in hippocampal neurogenesis. Biol Psychiatry 2006, 59:1087-96.

[58] Fanselow MS, Dong HW. Are the dorsal and ventral hippocampus functionally distinct structures? Neuron 2010, 65:7-19.

[59] Kempermann G, Kuhn HG, Gage FH. Genetic influence on neurogenesis in the dentate gyrus of adult mice. Proc Natl Acad Sci U S A 1997, 94:10409-14.

[60] van Praag H, Shubert T, Zhao C, Gage FH. Exercise enhances learning and hippocampal neurogenesis in aged mice. J Neurosci 2005, 25:8680-5.

[61] Warner-Schmidt JL, Duman RS. Hippocampal neurogenesis: opposing effects of stress and antidepressant treatment. Hippocampus 2006, 16: 239-49.

[62] Overstreet-Wadiche LS, Bromberg DA, Bensen AL, Westbrook GL. Seizures accelerate functional integration of adult-generated granule cells. J Neurosci 2006, 26:4095-103.

[63] Laplagne DA, Esposito MS, Piatti VC, Morgenstern NA, Zhao C, van Praag H, *et al*. Functional convergence of neurons generated in the developing and adult hippocampus. PLoS biology 2006, 4:409.

[64] Toni N, Teng EM, Bushong EA, Aimone JB, Zhao C, Consiglio A, *et al*. Synapse formation on neurons born in the adult hippocampus. Nat Neurosci 2007, 10:727-34. Epub 2007/05/09.

[65] Schmidt-Hieber C, Jonas P, Bischofberger J. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. Nature 2004, 429:184-7.

[66] Ge S, Yang CH, Hsu KS, Ming GL, Song H. A critical period for enhanced synaptic plasticity in newly generated neurons of the adult brain. Neuron 2007, 54:559-66.

[67] Faulkner RL, Jang MH, Liu XB, Duan X, Sailor KA, Kim JY, *et al*. Development of hippocampal mossy fiber synaptic outputs by new neurons in the adult brain. Proc Natl Acad Sci U S A 2008, 105:14157-62.

[68] Huang GJ, Bannerman D, Flint J. Chronic fluoxetine treatment alters behavior, but not adult hippocampal neurogenesis, in BALB/cJ mice. Mol Psychiatry 2008, 13:119-21.

[69] Meshi D, Drew MR, Saxe M, Ansorge MS, David D, Santarelli L, *et al*. Hippocampal neurogenesis is not required for behavioral effects of environmental enrichment. Nature neuroscience 2006, 9:729-31.

[70] Katz RJ. Animal model of depression: pharmacological sensitivity of a hedonic deficit. Pharmacol Biochem Behav 1982, 16:965-8.

[71] Willner P. Chronic mild stress [CMS] revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. Neuropsychobiology 2005, 52:90-110.

[72] Willner P, Towell A, Sampson D, Sophokleous S, Muscat R. Reduction of sucrose preference by chronic unpredictable mild stress, and its restoration by a tricyclic antidepressant. Psychopharmacology (Berl) 1987, 93:358-64.

[73] Surget A, Saxe M, Leman S, Ibarguen-Vargas Y, Chalon S, Griebel G, *et al*. Drug-dependent requirement of hippocampal neurogenesis in a model of depression and of antidepressant reversal. Biological Psychiatry 2008, 64:293-301.

[74] Nestler EJ, Gould E, Manji H, Buncan M, Duman RS, Greshenfeld HK, *et al*. Preclinical models: status of basic research in depression. Biological Psychiatry 2002, 52:503-28.

[75] Jayatissa MN, Bisgaard C, Tingstrom A, Papp M, Wiborg O. Hippocampal cytogenesis correlates to escitalopram-mediated recovery in a chronic mild stress rat model of depression. Neuropsychopharmacology 2006, 31:2395-404.

[76] Bisgaard CF, Jayatissa MN, Enghild JJ, Sanchez C, Artemychyn R, Wiborg O. Proteomic investigation of the ventral rat hippocampus links DRP-2 to escitalopram treatment resistance and SNAP to stress resilience in the chronic mild stress model of depression. J Mol Neurosci 2007, 32:132-44.

[77] Isingrini E, Camus V, Le Guisquet AM, Pingaud M, Devers S, Belzung C. Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: a model of fluoxetine resistance in mice. PLoS ONE 2010, 5:10404.

[78] Berton O, McClung CA, Dileone RJ, Krishnan V, Renthal W, Russo SJ, *et al*. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 2006, 311:864-8.

[79] Tsankova NM, Berton O, Renthal W, Kumar A, Neve RL, Nestler EJ. Sustained hippocampal chromatin regulation in a mouse model of depression and antidepressant action. Nat Neurosci 2006, 9:519-25.

[80] Krishnan V, Han MH, Graham DL, Berton O, Renthal W, Russo SJ, *et al*. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 2007, 131:391-404.

[81] Krishnan V, Nestler EJ. The molecular neurobiology of depression. Nature 2008, 455:894-902.

[82] Vialou V, Robison AJ, Laplant QC, Covington HE, 3rd, Dietz DM, Ohnishi YN, *et al*. DeltaFosB in brain reward circuits mediates resilience to stress and antidepressant responses. Nat Neurosci 2010, 13:745-52.

[83] de Kloet ER, Joels M, Holsboer F. Stress and the brain: from adaptation to disease. Nat Rev Neurosci 2005, 6:463-75.

[84] Antonijevic IA. Depressive disorders -- is it time to endorse different pathophysiologies? Psychoneuroendocrinology 2006, 31:1-15.

[85] Carroll BJ, Feinberg M, Greden JF, Tarika J, Albala AA, Haskett RF, *et al*. A specific laboratory test for the diagnosis of melancholia. Standardization, validation, and clinical utility. Arch Gen Psychiatry 1981, 38:15-22.

[86] Nemeroff CB, Widerlov E, Bissette G, Walleus H, Karlsson I, Eklund K, *et al*. Elevated concentrations of CSF corticotropin-releasing factor-like immunoreactivity in depressed patients. Science 1984, 226:1342-4.

[87] Sachar EJ, Hellman L, Fukushima DK, Gallagher TF. Cortisol production in depressive illness. A clinical and biochemical clarification. Arch Gen Psychiatry 1970, 23:289-98.

[88] Strohle A, Holsboer F. Stress responsive neurohormones in depression and anxiety. Pharmacopsychiatry 2003, 36:S207-14.

[89] Murray F, Smith DW, Hutson PH. Chronic low dose corticosterone exposure decreased hippocampal cell proliferation, volume and induced anxiety and depression like behaviours in mice. Eur J Pharmacol 2008, 583:115-27.

[90] David DJ, Samuels BA, Rainer Q, Wang J-W, Marsteller D, Mendez I, *et al*. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 2009, 62:479-93.

[91] Ardayfio P, Kim K-S. Anxiogenic-like effect of chronic corticosterone in the light-dark emergence task in mice. Behav Neurosci 2006, 120:249-56.

[92] Gourley SL, Wu FJ, Kiraly DD, Ploski JE, Kedves AT, Duman RS, *et al*. Regionally specific regulation of ERK MAP kinase in a model of antidepressant-sensitive chronic depression. Biological Psychiatry 2008, 63:353-9.

[93] Gourley SL, Wu FJ, Taylor JR. Corticosterone regulates pERK1/2 map kinase in a chronic depression model. Ann N Y Acad Sci 2008, 1148:509-14.

[94] Anacker C, Zunszain PA, Cattaneo A, Carvalho LA, Garabedian MJ, Thuret S, *et al*. Antidepressants increase human hippocampal neurogenesis by activating the glucocorticoid receptor. Mol Psychiatry 2011, 16:738-50.

[95] Perera TD, Dwork AJ, Keegan KA, Thirumangalakudi L, Lipira CM, Joyce N, *et al*. Necessity of hippocampal neurogenesis for the therapeutic action of antidepressants in adult nonhuman primates. PloS one 2011, 6:17600.

[96] Bessa JM, Ferreira D, Melo I, Marques F, Cerqueira JJ, Palha JA, *et al*. The mood-improving actions of antidepressants do not depend on neurogenesis but are associated with neuronal remodeling. Mol Psychiatry 2009, 14:764-73.

[97] David DJ, Samuels BA, Rainer Q, Wang JW, Marsteller D, Mendez I, *et al*. Neurogenesis-dependent and -independent effects of fluoxetine in an animal model of anxiety/depression. Neuron 2009, 62:479-93.

[98] Shors TJ, Leuner B. Estrogen-mediated effects on depression and memory formation in females. Journal of affective disorders 2003, 74:85-96.

[99] Dalla C, Papachristos EB, Whetstone AS, Shors TJ. Female rats learn trace memories better than male rats and consequently retain a greater proportion of new neurons in their hippocampi. Proc Natl Acad Sci U S A 2009, 106:2927-32.

[100] Schloesser RJ, Manji HK, Martinowich K. Suppression of adult neurogenesis leads to an increased hypothalamo-pituitary-adrenal axis response. Neuroreport 2009, 20:553-7.

[101] Snyder JS, Soumier A, Brewer M, Pickel J, Cameron HA. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. Nature 2011, 476:458-61.

[102] Dunn JD, Orr SE. Differential plasma corticosterone responses to hippocampal stimulation. Experimental brain research Experimentelle Hirnforschung Experimentation cerebrale 1984, 54:1-6.

[103] Herman JP, Figueiredo H, Mueller NK, Ulrich-Lai Y, Ostrander MM, Choi DC, *et al*. Central mechanisms of stress integration: hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. Frontiers in neuroendocrinology 2003, 24:151-80.

[104] Herman JP, Ostrander MM, Mueller NK, Figueiredo H. Limbic system mechanisms of stress regulation: hypothalamo-pituitary-adrenocortical axis. Progress in neuro-psychopharmacology & biological psychiatry 2005, 29:1201-13.

[105] Jankord R, Herman JP. Limbic regulation of hypothalamo-pituitary-adrenocortical function during acute and chronic stress. Annals of the New York Academy of Sciences 2008, 1148:64-73.

[106] Snyder JS, Ramchand P, Rabbett S, Radik R, Wojtowicz JM, Cameron HA. Septo-temporal gradients of neurogenesis and activity in 13-month-old rats. Neurobiol Aging 2011, 32:1149-56.

[107] Sahay A, Drew MR, Hen R. Dentate gyrus neurogenesis and depression. Prog Brain Res 2007, 163:697-722.

[108] Sahay A, Hen R. Adult hippocampal neurogenesis in depression. Nat Neurosci 2007, 10:1110-5.

[109] Moser E, Moser MB, Andersen P. Synaptic potentiation in the rat dentate gyrus during exploratory learning. Neuroreport 1993, 5:317-20.

[110] Bannerman DM, Rawlins JN, McHugh SB, Deacon RM, Yee BK, Bast T, *et al*. Regional dissociations within the hippocampus--memory and anxiety. Neuroscience and biobehavioral reviews 2004, 28:273-83.

[111] Ming GL, Song H. Adult neurogenesis in the mammalian central nervous system. Annual review of neuroscience 2005, 28:223-50.

[112] Li Y, Mu Y, Gage FH. Development of neural circuits in the adult hippocampus. Current topics in developmental biology 2009, 87:149-74.

[113] Lacefield CO, Itskov V, Reardon T, Hen R, Gordon JA. Effects of adult-generated granule cells on coordinated network activity in the dentate gyrus. Hippocampus 2012, 22:106-16.

[114] Bergami M, Rimondini R, Santi S, Blum R, Gotz M, Canossa M. Deletion of TrkB in adult progenitors alters newborn neuron integration into hippocampal circuits and increases anxiety-like behavior. Proc Natl Acad Sci U S A 2008, 105:15570-5.

[115] Shors TJ, Townsend DA, Zhao M, Kozorovitskiy Y, Gould E. Neurogenesis may relate to some but not all types of hippocampal-dependent learning. Hippocampus 2002, 12:578-84.

[116] Noonan MA, Bulin SE, Fuller DC, Eisch AJ. Reduction of adult hippocampal neurogenesis confers vulnerability in an animal model of cocaine addiction. J Neurosci 2010, 30:304-15.

[117] Surget A, Tanti A, Leonardo ED, Laugeray A, Rainer Q, Touma C, *et al*. Antidepressants recruit new neurons to improve stress response regulation. Mol Psychiatry 2011, 16:1177-88.

[118] Jiang W, Zhang Y, Xiao L, Van Cleemput J, Ji SP, Bai G, *et al*. Cannabinoids promote embryonic and adult hippocampus neurogenesis and produce anxiolytic- and antidepressant-like effects. The Journal of clinical investigation 2005, 115:3104-16.

[119] Jaholkowski P, Kiryk A, Jedynak P, Ben Abdallah NM, Knapska E, Kowalczyk A, *et al*. New hippocampal neurons are not obligatory for memory formation; cyclin D2 knockout mice with no adult brain neurogenesis show learning. Learn Mem 2009, 16:439-51.

[120] Cowen DS, Takase LF, Fornal CA, Jacobs BL. Age-dependent decline in hippocampal neurogenesis is not altered by chronic treatment with fluoxetine. Brain Res 2008, 1228:14-9.

[121] Kodama M, Fujioka T, Duman RS. Chronic olanzapine or fluoxetine administration increases cell proliferation in hippocampus and prefrontal cortex of adult rat. Biol Psychiatry 2004, 56:570-80.

[122] Nasrallah HA, Hopkins T, Pixley SK. Differential effects of antipsychotic and antidepressant drugs on neurogenic regions in rats. Brain Res 2010, 1354:23-9.

[123] Marcussen AB, Flagstad P, Kristjansen PE, Johansen FF, Englund U. Increase in neurogenesis and behavioural benefit after chronic fluoxetine treatment in Wistar rats. Acta neurologica Scandinavica 2008, 117:94-100.

[124] Huang GJ, Herbert J. Stimulation of neurogenesis in the hippocampus of the adult rat by fluoxetine requires rhythmic change in corticosterone. Biol Psychiatry 2006, 59:619-24.

[125] Sairanen M, Lucas G, Ernfors P, Castren M, Castren E. Brain-derived neurotrophic factor and antidepressant drugs have different but coordinated effects on neuronal turnover, proliferation, and survival in the adult dentate gyrus. J Neurosci 2005, 25:1089-94.

[126] Manev R, Uz T, Manev H. Fluoxetine increases the content of neurotrophic protein S100beta in the rat hippocampus. European journal of pharmacology 2001, 420:R1-2.

[127] Miller BH, Schultz LE, Gulati A, Cameron MD, Pletcher MT. Genetic regulation of behavioral and neuronal responses to fluoxetine. Neuropsychopharmacology 2008, 33:1312-22.

[128] Navailles S, Hof PR, Schmauss C. Antidepressant drug-induced stimulation of mouse hippocampal neurogenesis is age-dependent and altered by early life stress. J Comp Neurol 2008, 509:372-81.

[129] Kong H, Sha LL, Fan Y, Xiao M, Ding JH, Wu J, *et al*. Requirement of AQP4 for antidepressive efficiency of fluoxetine: implication in adult hippocampal neurogenesis. Neuropsychopharmacology 2009, 34:1263-76.

[130] Bai F, Bergeron M, Nelson DL. Chronic AMPA receptor potentiator (LY451646) treatment increases cell proliferation in adult rat hippocampus. Neuropharmacology 2003, 44:1013-21.

[131] Yoshimizu T, Chaki S. Increased cell proliferation in the adult mouse hippocampus following chronic administration of group II metabotropic glutamate receptor antagonist, MGS0039. Biochemical and biophysical research communications 2004, 315:493-6.

[132] Koo JW, Duman RS. Interleukin-1 receptor null mutant mice show decreased anxiety-like behavior and enhanced fear memory. Neurosci Lett 2009, 456:39-43.

[133] Jaako-Movits K, Zharkovsky T, Pedersen M, Zharkovsky A. Decreased hippocampal neurogenesis following olfactory bulbectomy is reversed by repeated citalopram administration. Cellular and molecular neurobiology 2006, 26:1559-70.

[134] Ekstrand J, Hellsten J, Wennstrom M, Tingstrom A. Differential inhibition of neurogenesis and angiogenesis by corticosterone in rats stimulated with electroconvulsive seizures. Progress in neuro-psychopharmacology & biological psychiatry 2008, 32:1466-72.

[135] Keilhoff G, Becker A, Grecksch G, Bernstein HG, Wolf G. Cell proliferation is influenced by bulbectomy and normalized by imipramine treatment in a region-specific manner. Neuropsychopharmacology 2006, 31:1165-76.

[136] Silva R, Mesquita AR, Bessa J, Sousa JC, Sotiropoulos I, Leao P, *et al*. Lithium blocks stress-induced changes in depressive-like behavior and hippocampal cell fate: the role of glycogen-synthase-kinase-3beta. Neuroscience. 2008, 152:656-69.

[137] Chen G, Masana MI, Manji HK. Lithium regulates PKC-mediated intracellular cross-talk and gene expression in the CNS *in vivo*. Bipolar disorders 2000, 2:217-36. Epub 2001/03/16.

[138] Katoh-Semba R, Asano T, Ueda H, Morishita R, Takeuchi IK, Inaguma Y, *et al*. Riluzole enhances expression of brain-derived neurotrophic factor with consequent proliferation of granule precursor cells in the rat hippocampus. FASEB journal : official publication of the Federation of American Societies for Experimental Biology 2002, 16:1328-30.

[139] Nakagawa S, Kim JE, Lee R, Malberg JE, Chen J, Steffen C, *et al*. Regulation of neurogenesis in adult mouse hippocampus by cAMP and the cAMP response element-binding protein. The Journal of neuroscience : the official journal of the Society for Neuroscience 2002, 22:3673-82.

[140] Larsen MH, Rosenbrock H, Sams-Dodd F, Mikkelsen JD. Expression of brain derived neurotrophic factor, activity-regulated cytoskeleton protein mRNA, and enhancement of adult hippocampal neurogenesis in rats after sub-chronic and chronic treatment with the triple monoamine re-uptake inhibitor tesofensine. European journal of pharmacology 2007, 555:115-21.

[141] Czeh B, Michaelis T, Watanabe T, Frahm J, de Biurrun G, van Kampen M, *et al*. Stress-induced changes in cerebral metabolites, hippocampal volume, and cell proliferation are prevented by antidepressant treatment with tianeptine. Proc Natl Acad Sci U S A 2001, 98:12796-801.

[142] Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. The Journal of neuroscience : the official journal of the Society for Neuroscience 2000, 20:9104-10.

[143] Mostany R, Valdizan EM, Pazos A. A role for nuclear beta-catenin in SNRI antidepressant-induced hippocampal cell proliferation. Neuropharmacology 2008, 55:18-26.