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 thymidine, 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 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 serotoninergic 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.
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 I):

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
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 neuronal 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

<table>
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<th>Antidepressant</th>
<th>Specie</th>
<th>Dose (mg/kg)</th>
<th>Length of Treatment</th>
<th>Prolif.</th>
<th>Diff.</th>
<th>Survival</th>
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<td>Rodents</td>
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Legend: ↑, increase; ↓, decrease; =, no change; ø, not study
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<th>Survival</th>
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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 refractory 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 glucocortioid 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 neurogenesis 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.

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