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Implications of the Functional Integration of Adult-Born Hippocampal Neurons in Anxiety-Depression Disorders

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Abstract

Adult neurogenesis in the dentate gyrus of the hippocampus has gained considerable attention as a cellular substrate for both the pathophysiology and treatment of depression. Overall, the studies of adult hippocampal neurogenesis are still in their infancy because most of them explore only one stage of this process. Importantly, given the built-in homeostatic mechanisms that act at each stage during the progression from stem cells to mature neurons (proliferation, differentiation, maturation, survival), it is very difficult to extrapolate the efficiency of a drug on adult neurogenesis from analysis of one stage alone. Here, we review the most significant data on hippocampal neurogenesis, focusing on the importance of studying each stage of adult hippocampal neurogenesis and also on the importance of choosing the appropriate mouse strain to perform the experiment. Specifically, strains with a high number of basal proliferating cells in the dentate gyrus of the hippocampus should be used only under stressed conditions to detect the effects of antidepressants on adult neurogenesis. We also discuss how adult hippocampal neurogenesis could be involved in affective state disorders such as depression and anxiety. Finally, we reveal that the behavioral effects of fluoxetine are mediated through both neurogenesis-dependent and -independent actions.

Keywords

adult neurogenesis, antidepressant, corticosterone model, hippocampus, mice, neurogenesis dependent, neurogenesis independent, strain

Mood disorders impact 7% of the world's population, and severe forms of depression affect 2% to 5% of the US population (Kessler and others 2005). The diagnostic criteria of major depressive disorder (MDD) includes the persistence of depressed mood, low self-esteem, feelings of hopelessness, anhedonia, decreased ability to concentrate, abnormalities in appetite, neurovegetative symptoms, weight loss or gain, insomnia or hypersomnia, and recurrent thoughts of suicide (American Psychiatric Association 1990). The heterogeneous nature of depression suggests an involvement of multiple distinct brain regions, which may be responsible for the diverse symptoms. Human imaging and postmortem studies have supported this hypothesis, implicating brain areas including the prefrontal and cingulate cortex, hippocampus, striatum, amygdala, and thalamus (Drevets 2001; Liotti and Mayberg 2001; Nestler and others 2002). Together, these brain regions operate a series of highly interacting circuits that forms a neural circuitry involved in depression (Drevets 2001; Manji and others 2001; Nestler and others 2002).

The hippocampus is one of several limbic structures that has been extensively studied in individuals with psychiatric and neurological disorders in the last decade (Eisch and others 2008). Besides its critical role in learning and memory, the hippocampus is one of only 2 areas in the mammalian brain in which adult neurogenesis

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occurs (Eisch and others 2008). This physiological phenomenon can be divided into discrete stages, each of which is defined by distinct physiological and morphological properties (Sahay and Hen 2007). Birth of new neurons, or neurogenesis, occurs throughout life, specifically in 2 areas of the brain in adult mammals: the subventricular zone (SVZ) of the lateral ventricles and the subgranular zone (SGZ) of the dentate gyrus (DG) of the hippocampus. New neurons born in the SGZ migrate into the granule cell layer of the DG and eventually become granule cells. These newborn neurons integrate into the existing circuitry and receive functional input (Zhao and others 2008). Adult neurogenesis in the hippocampus is therefore defined as the progression from neural stem cell to mature dentate granule neuron. All stages of adult neurogenesis are regulated by physiological activity, including the proliferation, differentiation, fate determination of adult neural stem cells (NSCs) and progenitors, and the survival, maturation, and integration of newborn neurons (Zhao and others 2008). 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, one deserves particular attention because it allows the characterization of changes in the brain following chronic but not acute antidepressant treatments: the neurogenesis hypothesis of depression. 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 nonstressed and stressed rodents.

Quantitative Analysis of Proliferation, Survival, Maturation, and Differentiation of 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. Proliferation, differentiation, and survival steps require a specific protocol using the administration of a synthetic thymidine analog, "5-bromo-3'-deoxyuridine" (BrdU), which 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 (Kempermann and others 1997; Miller and Nowakowski 1988) (Fig. 1A, 1E). Usually, for the quantification of number of rate of cell division, animals are administered BrdU 2 hours before sacrifice (Taupin 2007) (Fig. 1A). The fate of the newly generated cells can be determined 3 to 4 weeks later once migration has been achieved (Cameron and others 1995; Paizanis and others 2007) (Fig. 1E). Proliferation, differentiation, or survival is quantified by counting BrdU-positive cells (Fig. 1D, 1F). Interestingly, quantification of the BrdU-positive clusters could also be performed to measure proliferation because a positive correlation exists between BrdU-positive clusters and BrdU-positive cells (Fig. 1C). Because 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. 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.

Anxiety/Depressive-Like State and Adult Hippocampal Neurogenesis

The neurogenesis hypothesis of depression postulates that a decrease in the production of newborn granule cells in the dentate gyrus of the hippocampus is related to the pathophysiology of depression and that enhanced hippocampal neurogenesis is required for the behavioral effect of antidepressant treatments (Sheline and others 1996). However, the study of adult hippocampal neurogenesis in depressed patients has relied primarily on histological examination of postmortem brain tissue and magnetic resonance imaging studies. The only study to date did not detect a difference in proliferation of stem cells (using KI-67) in the hippocampus of depressed patients (Reif and others 2006). Although notable, the study is limited in power and confounded by the effects of medication (all the depressed subjects studied, with the exception of one, were prescribed antidepressant medications at time of death) that may have masked small differences in cell proliferation. Moreover, no toxicology data were available for those subjects, and thus, it is not known whether the medications prescribed were actually taken by the patient. More importantly, given the built-in homeostatic mechanisms that act at each stage of progression from stem cell to mature neuron, it is very difficult to extrapolate from analysis of one stage alone.

Magnetic resonance imaging studies have been also used to investigate whether impaired adult neurogenesis is an etiological factor for depression. A reduction in hippocampal volume in depressed patients has been consistently shown, and 2 meta-analyses have compellingly demonstrated a reduction in hippocampal volume relative to age- and sex-matched controls in people with

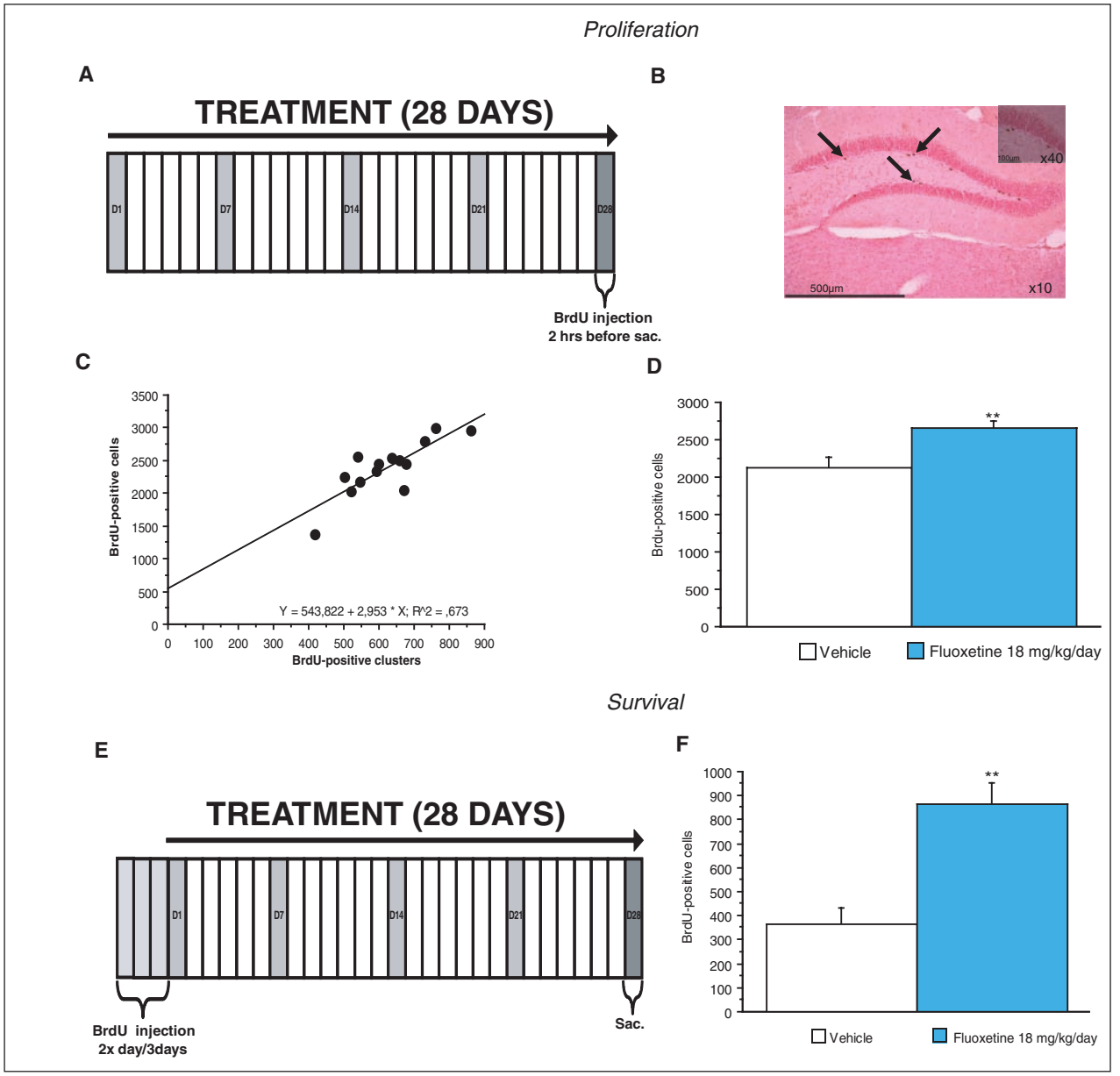

Figure 1. Experimental protocol to assess the effects of monoaminergic antidepressant treatment on proliferation and survival steps of adult hippocampal neurogenesis in 129SvEv strain. (A) To assess the effects of chronic fluoxetine treatment (28 days of treatment) on cell proliferation, "5-bromo-3'-deoxyuridine" (BrdU) (150 mg/kg) is administered 2 hours before sacrifice. (B) Photograph of BrdU-positive clusters in the dentate gyrus of adult hippocampus. BrdU-positive cell counts for the subgranular zone and adjacent zone are defined as a 2–cell body–wide zone along the hilar border (10× magnification). (C) A positive correlation between BrdU-positive clusters and BrdU-positive cells is observed (R^2 = 0.67). The quantification of BrdU-positive clusters could be used as a rapid indicator of the neurogenic effect of drugs. (D) Immunohistochemistry of cell proliferation of newborn cells in the dentate gyrus of the hippocampus indicates a significant enhancement of BrdU-positive cells after chronic fluoxetine treatment (18 mg/kg/d) in 129SvEv mice. Values plotted are mean ± SEM (n = 7 per group). $^{**}P < 0.01$ versus control group. Data were analyzed using StatView 5.0 software (SAS Institute, Cary, NC). One-way ANOVA was applied to the data as appropriate ($F_{1,12}$ = 8.96; $^{**}P < 0.01$), followed by Fisher protected least significant difference (PLSD) post hoc analysis. (E) To assess the effects of chronic fluoxetine treatment (28 days of treatment) on survival of newborn neurons, BrdU (150 mg/kg) is administered for 3 days, twice a day, before the start of the treatment. Animals are sacrificed at the end of the chronic treatment. (F) Immunohistochemistry of survival of newborn cells in the dentate gyrus of the hippocampus indicates a significant enhancement of BrdU-positive cells after chronic fluoxetine treatment (18 mg/kg/d) in 129SvEv mice. Values plotted are mean ± SEM (n = 9 per group). $^{**}P < 0.01$ versus control group. Data were analyzed using StatView 5.0 software (SAS Institute). One-way ANOVA was applied to the data as appropriate ($F_{1,16}$ = 12.88; $^{**}P < 0.01$), followed by Fisher PLSD post hoc analysis.

recurrent depression (Videbech and Ravnkilde 2004). The frequency of depressive episodes and how long the depression remains untreated correlate with the magnitude of reduction in hippocampal volume. However, pathohistological studies of postmortem tissue indicate that changes in neuropil and glial cell number may be responsible for reductions in hippocampal volume (Czeh and Lucassen 2007).

Preclinical studies have proven to be informative in bridging the causality between adult hippocampal neurogenesis and behavior. Using exposure to different forms of chronic stress, such as social subordination, immobilization, physical restraint, and foot shock, a decrease in SGZ proliferation in rodents has been observed (Gould, McEwen and others 1997; Gould, Tanapat and others 1998). However, the dissection of the causal relationship between hippocampal neurogenesis and behavior came from ablation of progenitor cells. Several methods have been developed to decrease or ablate neurogenesis, including 1) low-dose X-ray or gamma-ray irradiation of either the whole brain or restricted brain regions (Santarelli and others 2003); 2) systemic treatment with antimitotic drugs such as methylazoxymethanol acetate (MAM) (Jayatissa and others 2009); and 3) genetically manipulated mice to specifically ablate neurogenesis, such as the GFAP-TK mice in which dividing GFAP+ progenitors are susceptible to ganciclovir treatment (Saxe and others 2006). It is important to keep in mind the drawbacks of these methods, such as nonspecific effects of ablation that could involve not only the hippocampus but also other brain regions and functions and the lack of temporal specificity of ablation. Impaired adult neurogenesis in the hippocampus was hypothesized to be a part of the pathogenesis of major depressive disorders (Duman and others 2000; Kempermann and Kronenberg 2003). Blocking hippocampal neurogenesis using X-irradiation or genetic ablation (GFAP-TK mice) does not influence anxiety-related behavior as assessed in conflict-based tests, such as the open field, light-dark choice test, and elevated plus maze or in anxiety tests that are also used to screen for antidepressant activity, such as novelty-suppressed feeding (Santarelli and others 2003; Saxe and others 2006; David and others 2007). Moreover, the X-irradiation of the hippocampus per se had no effect on stress, suggesting that a loss of hippocampal neurogenesis is not sufficient to induce anxiety/depressive-like behavior and does not worsen the behavioral changes induced by stress. Similarly, using a pharmacological ablation of cell proliferation with MAM, it was demonstrated that suppression of cell proliferation in the hippocampal formation is not an absolute factor for induction of an anhedonia-like state in rats (Jayatissa and others 2009). Thus, it seems that a decrease in neurogenesis is not sufficient to mediate the development of an anxiety/depressive-like state in rodents. However, further studies suggested that the situation is more complicated. Recently, results indicate that adult hippocampal neurogenesis plays an important role in the regulation of affective states (Revest and others 2009). In this transgenic model, adult hippocampal neurogenesis is selectively impaired by overexpression of the proapoptotic protein Bax in neuronal precursors. Using several behavioral paradigms, authors showed that a deficit in hippocampal neurogenesis increased anxiety-related behaviors but did not modify behaviors that are related to the affective sphere underlying depression. Finally, Airan and others (2007) further explored the potential link between depression and neurogenesis using voltage-sensitive dye imaging to probe hippocampal activity. Intriguingly, against the neurogenesis hypothesis of depression, chronic stress in rats was not associated with a down-regulation in neurogenesis, and ablation of neurogenesis did not induce a depression-like state (Airan and others 2007). In summary, current evidence indicates that adult hippocampal neurogenesis may not be a major contributor to the development of depression but may be required for some of the behavioral effects of antidepressants (Sahay and Hen 2007).

Antidepressant Effects on Proliferation, Differentiation, Maturation, and Survival on Stages in Adult Nonstressed Animals

One of the primary focuses for the role of adult hippocampal neurogenesis in depression is the observation that antidepressants and environmental interventions that confer antidepressant-like behavioral effects stimulate adult hippocampal neurogenesis in rodents and in humans. A recent elegant study showed the first evidence that in the human dentate gyrus, there are more neuronal progenitor cells (Nestin-Immunoreactive) and more dividing cells (Ki-67-Immunoreactive) in selective serotonin reuptake inhibitor (SSRI) (sertraline, fluoxetine)– or tricyclic antidepressant (TCA) (nortriptyline, clomipramine)–treated MDD patients compared with untreated MDD or controls (Boldrini and others 2009).

Antidepressant Effects on Proliferation or Survival Stages

In this review, we replicated previous data showing that chronic fluoxetine (18 mg/kg/d) treatment and other monoaminergic antidepressants increased proliferation of progenitor cells in 129SvEv mice (Santarelli and others 2003) (Fig. 1D). Moreover, aside from increasing proliferation, fluoxetine also enhanced the survival of postmitotic granule cells (Fig. 1F). The

effects of monoaminergic antidepressants on cell proliferation and survival of newborn neurons have also been demonstrated in rats (Encinas and others 2006; Malberg and others 2000). Interestingly, the effects of monoaminergic antidepressants on proliferation and survival were observed after chronic but not subchronic treatment (Malberg and others 2000; Duman and others 2001; Santarelli and others 2003; David and others 2007; Wang and others 2008). Furthermore, other antidepressants such as atypical antidepressant tianeptine, electroconvulsive therapy, mood stabilizers, and the novel antidepressant agomelatine (a mixed MT1/MT2 melatonin receptor agonist and 5-HT2C receptor antagonist) also increased proliferation and survival stages in the adult hippocampus (Chen and others 2006; Banasr and others 2006). Interestingly, it was shown recently that antidepressants could differentially affect various stages of neurogenesis in the dorsal and ventral hippocampus. For example, chronic (3 weeks) administration of agomelatine increased cell proliferation and survival in the ventral dentate gyrus, a region notably implicated in response to emotion (Banasr and others 2006).

Studies have suggested that distinct mechanisms regulate proliferation and survival. For example, environmental enrichment enhances the survival of immature cells without affecting proliferation (Kempermann and others 1997). In contrast, voluntary exercise increases proliferation and survival but does not alter the rate of maturation (Plumpe and others 2006) or dendritic morphology of newborn neurons (van Praag and others 2005). Pilocarpine-induced seizures cause both increased proliferation and survival (Radley and Jacobs 2003), along with improved dendritic outgrowth in newborn neurons (Overstreet-Wadiche and others 2006). Finally, a recent study has shown that fluoxetine targets a class of amplifying neural progenitors by increasing the rate of symmetric divisions (Encinas and others 2006).

Antidepressant Effects on Maturation Stage

Until recently, it was not clear whether SSRIs also target immature neurons by influencing their maturation and functional integration. We showed here (Fig. 2) and also recently (Wang and others 2008) that chronic fluoxetine increases proliferation of progenitors and also survival of immature neurons in the adult dentate gyrus of the hippocampus, which is consistent with several previous studies (Encinas and others 2006; Malberg and others 2000; Santarelli and others 2003; Soumier and others 2009). We have demonstrated for the first time that chronic, but not subchronic, fluoxetine administration stimulates maturation of immature granule cells: first, a larger fraction of doublecortin-positive (DCX+) cells possessed tertiary dendrites following chronic fluoxetine treatment; and second, these immature DCX+ cells displayed more complex dendritic arborization following chronic fluoxetine (Fig. 3B). Overall, newborn neurons undergo an accelerated maturation after chronic fluoxetine treatment, as shown by the increased proportion of newborn cells that ceased to express the immature neuronal marker DCX. The delayed effects of fluoxetine to stimulate maturation of young granule cells parallel the delayed onset of its behavioral effects. Interestingly, electroconvulsive therapy (ECT), one of the fastest and most effective antidepressant treatments (American Psychiatric Association 1990), stimulates neurogenesis more rapidly than fluoxetine (Warner-Schmidt and Duman 2007). In addition, the induction of seizures, a prerequisite for achieving therapeutic effects during ECT (American Psychiatric Association 1990), stimulates dendritic development and maturation (Overstreet-Wadiche and others 2006). Specifically, following seizure induction, newborn granule cells display increased dendritic outgrowth and start receiving glutamatergic synaptic input earlier than those from noninduced animals (Overstreet-Wadiche and others 2006). These studies, together with our results, suggest that the processes that promote the maturation of newborn cells, such as agomelatine, may be targets for future drug development (Soumier and others 2009).

Antidepressant Effects on Differentiation Stage

Adult NSCs, or neural progenitors, can differentiate into neurons and glial cells (astrocytes and oligodendrocytes) (Gage 2000). There are 2 types of neural progenitors in the SGZ: type 1 progenitors have a radial process spanning the granule cell layer, expressing nestin, glial fibrillary acidic protein (GFAP), and the Sry-related HMG box transcription factor, Sox2 (Fukuda and others 2003; Garcia and others 2004; Suh and others 2007). These type 1 cells are sometimes referred to as NSCs (neural stem cells). Type 2 hippocampal progenitors have only short processes and express Sox-2 but not GFAP (Zhao and others 2008) and are sometimes referred to as transit-amplifying cells or intermediate progenitors. Four weeks after birth, newly generated granule cells have acquired the typical features of mature granule cells; for example, newborn cells have ceased to express immature neuronal markers such as DCX or polysialated neural cell adhesion molecule (PSA-NCAM), and they receive similar glutamatergic and GABAergic inputs as existing mature neurons in the dentate gyrus (Laplagne and others 2006; Toni and others 2007; Zhao and others 2006). However, newborn cells continue to mature

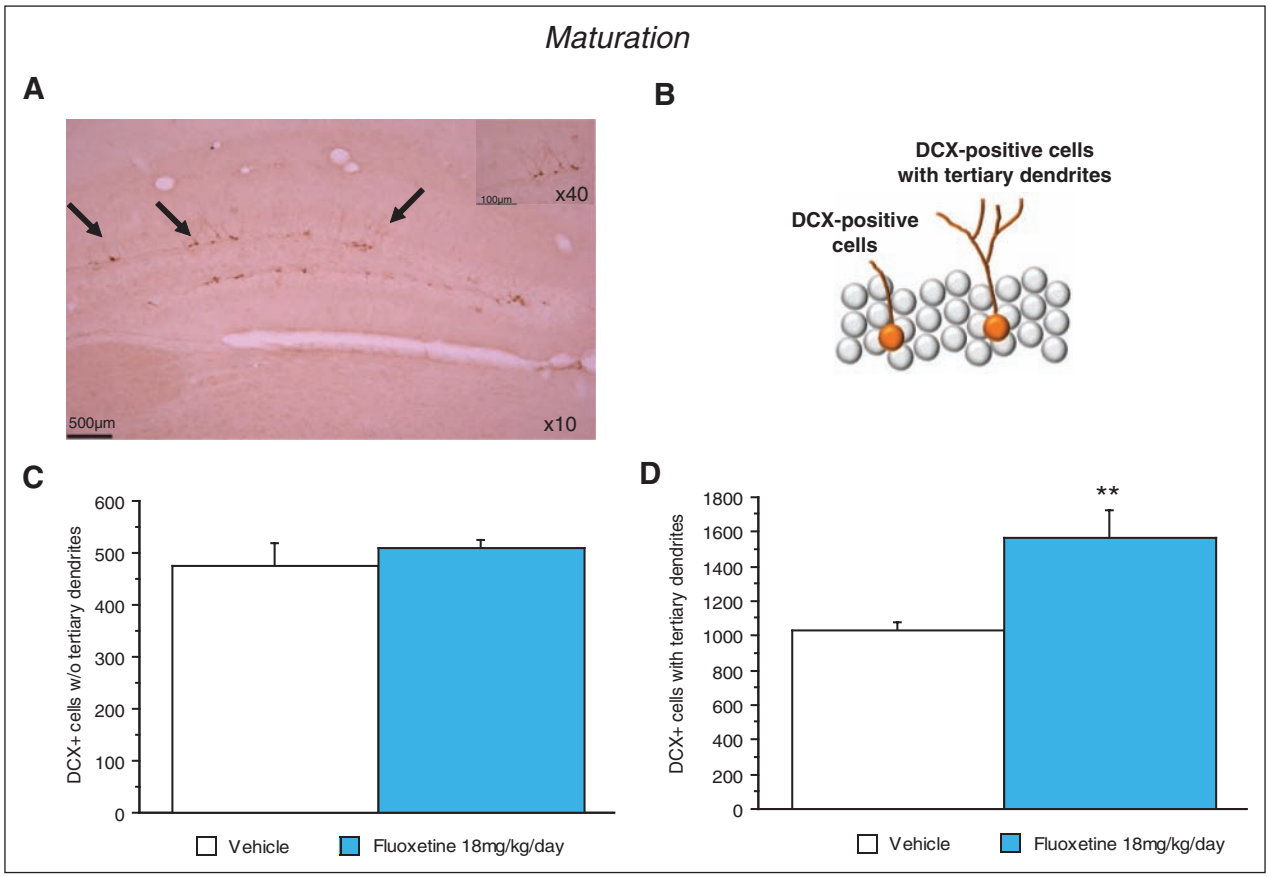

Figure 2. Chronic monoaminergic antidepressant stimulates dendritic maturation in 129SvEv strain. (A) Doublecortin (DCX) images were taken at 10× magnification and 40× magnification for the inset. (B) Categorization of DCX+ immature cells. We categorized DCX+ cells according to their dendritic morphology into DCX+ cells without tertiary dendrites and DCX+ cells with tertiary dendrites. (C) Chronic fluoxetine treatment (18 mg/kg/d) in 129SvEv strain did not change the total number of DCX+ cells. Values plotted are mean ± SEM (n = 4-5 per group). Data were analyzed using StatView 5.0 software (SAS Institute, Cary, NC). One-way ANOVA was applied to the data as appropriate ($F_{1,7}$ = 0.78). (D) Chronic fluoxetine treatment (18 mg/kg/d) in 129SvEv strain significantly increased the number of DCX+ cells with tertiary dendrites. Values plotted are mean ± SEM (n = 4-5 per group). $^{**}P < 0.01$ versus control group. Data were analyzed using StatView 5.0 software (SAS Institute). One-way ANOVA was applied to the data as appropriate ($F_{1,7}$ = 8.06; $^{**}P < 0.01$), followed by Fisher protected least significant difference post hoc analysis.

morphologically and physiologically. The spines of 4-week-old neurons are more likely to be associated with multiple-synapse boutons than older neurons, and the density of mushroom spines continues to increase after 8 weeks (Laplagne and others 2006). Furthermore, 2- to 4-week-old neurons display enhanced excitability and low long-term potentiation (LTP) induction threshold, whereas 4- to 6-week-old neurons display larger LTP amplitude (Schmidt-Hieber and others 2004). In addition, a form of LTP (ACSF-LTP) acquired using field recordings in the dentate gyrus has been shown to require hippocampal neurogenesis: ACSF-LTP is completely blocked by ablation of neurogenesis with either irradiation or a genetic manipulation (Saxe and others 2006). This critical period for the young neurons coincides with the developmentally regulated expression of NR2B-containing NMDARs in adult-born neurons (Tashiro and others 2007; Ge and others 2008).

Following differentiation, newborn neurons go through several developmental stages with distinctive physiological and morphological characteristics. Similar to newborn neurons in the developing brain, adult-born granule cells less than 3 weeks old depolarize in response to GABA because of their high intracellular chloride concentrations (Ge and others 2006). At 2 to 4 weeks after birth, the response to GABA switches from depolarization to hyperpolarization, the same period during which the growth of dendritic spines and the onset of glutamatergic

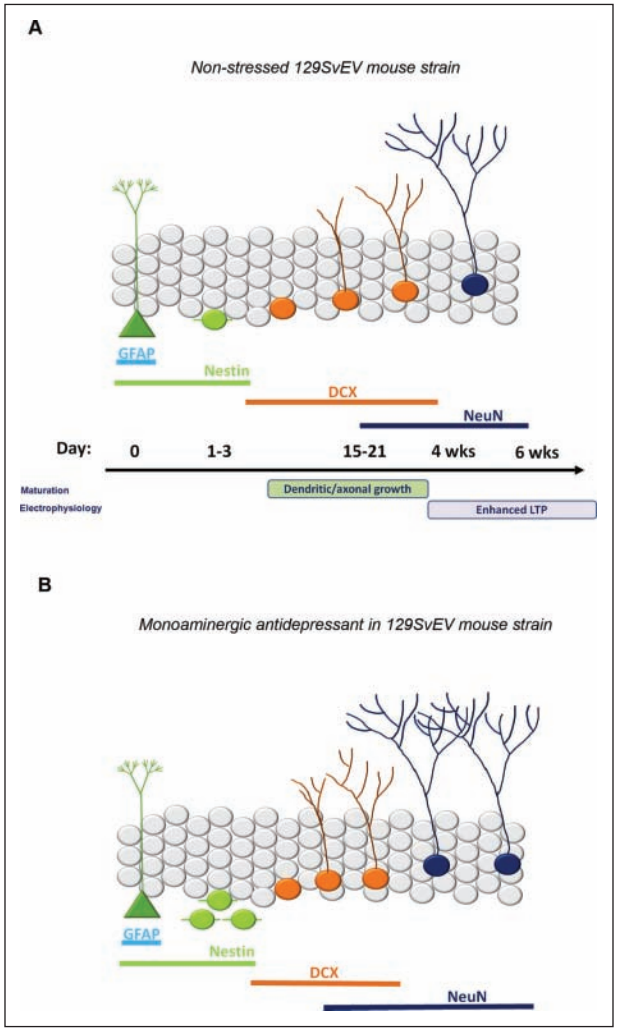

Figure 3. Chronic fluoxetine stimulates dendritic maturation and synaptic plasticity of newborn granule cells, a possible mechanism for antidepressant action in 129SvEv mice. (A, B) From left to right, anatomical and functional stages during neuronal differentiation and maturation, including quiescent, positive radial glia-like progenitors (green), rapidly amplifying neural progenitors (light green), immature granule cells (orange), and mature granule cells. Bottom panels show immunohistochemical markers for each stage (A). Fluoxetine stimulates adult neurogenesis in a multifold fashion in 129SvEv mice (B). Chronic fluoxetine treatment: first, increases proliferation of neural progenitors; second, stimulates dendritic branching as well as facilitates maturation; third, enhances survival of immature granule cells; and fourth, enables young neurons to be functionally integrated into the local hippocampal circuit, resulting in an enhancement of long-term synaptic plasticity (Wang and others 2008). Finally, these synergistic actions lead to an improved behavior outcome (Encinas and others 2006; Malberg and others 2000). Reprinted from Wang and others 2008, with permission of Society for Neuroscience.

input occurs (Ge and others 2006). In addition, newly generated granule cells form synaptic contacts with hilar and CA3 targets at 2 weeks of age, while the complexity of the synapses increases as neurons mature (Faulkner and others 2008).

Influence of Mouse Strain on Effects of Antidepressant on Adult Hippocampal Neurogenesis

Studies on different strains of mice yielded contrasting results with regard to the effects of antidepressant drugs on adult hippocampal neurogenesis (Table 1). For example, previous findings showed that in the 129SvEv strain, fluoxetine increases cell proliferation and survival of newborn cells (Santarelli and others 2003), whereas in BALB/cJ, it did not (Holick and others 2008). A recent report confirmed these data, indicating that neurogenesis may not always be required for the behavioral effects of fluoxetine, at least in the BALB/cJ strain (Huang and others 2008). For these reasons, the mouse strain might be an important factor, potentially accounting for the conflicting results of neurogenesis studies. It is possible that the 2 strains utilize different cellular and molecular machinery to mediate the neurogenic and behavioral effects of chronic antidepressant treatment. It has been examined whether strain differences exist in baseline or stress-induced changes in cell proliferation and survival in the dentate gyrus. Recent reports show that female mice of the BALB/c and a 129 substrain (129SvJ) are quite similar on measures of basal adult neurogenesis including cell proliferation, survival, and neuronal differentiation, although the 129SvJ mice show slightly less survival 4 weeks after BrdU injection (Kempermann and others 1997). Moreover, quantitative assessments of progenitor cell proliferation and immature neuronal differentiation in the dentate gyrus revealed significantly different basal proliferation rates between BALB/cJ and C57Bl/6 strains (2-fold more proliferating cells in C57Bl/6 than in BALB/cJ) (Navailles and others 2008). While neither of these strains responded to chronic antidepressant during adulthood, chronic stress unveiled the effects (Table 1). Finally, to study the effects of antidepressant in nonstressed animals, the choice of a strain is crucial. It is noteworthy that a strain such as 129SvEv mice, exhibiting low numbers of basal proliferating cells within the subgranular zone, is more appropriate in nonstressed conditions than BALB/cJ mice (Holick and others 2008) or C57BL/6 strain (Figs. 3A and 4A). On the contrary, the use of a strain exhibiting a high number of basal proliferating cells, such as C57BL/6 mice, would be better to study the impact of the stress on adult hippocampal neurogenesis, even though the action of antidepressants on neurogenesis is decreasing as a function of age (Couillard-Despres and others 2009).

Table 1. Effects of a Chronic Monoaminergic Antidepressant Treatment on Adult Hippocampal Neurogenesis Stages in Various Mouse Strains and under Various Experimental Conditions

Mouse Strain	Conditions	Proliferation	Survival	Maturation
129SvEv	Antidepressant treatment in nonstressed adult animals	↑ (Santarelli and others 2003; David and others 2007; Wang and others 2008)	↑ (Santarelli and others 2003; Wang and others 2008)	↑ (Santarelli and others 2003; Wang and others 2008)
BALB/cJ	Antidepressant treatment in nonstressed adult animals	No effect (Holick and others 2008; Huang and others 2008; Navailles and others 2008)	No effect (Huang and others 2008; Navailles and others 2008)	No effect (Holick and others 2008; Huang and others 2008; Navailles and others 2008)
	Antidepressant treatment during adolescence	↑ (Navailles and others 2008)	↑ (Navailles and others 2008)	↑ (Navailles and others 2008)
	Antidepressant treatment during early life stress	No effect (Navailles and others 2008)	No effect (Navailles and others 2008)	No effect (Navailles and others 2008)
	Antidepressant treatment during unpredictable chronic mild stress	↑ (Surget and others 2008)	Not tested (Surget and others 2008)	Not tested (Surget and others 2008)
C57BL/6	Antidepressant treatment in nonstressed adult animals	No effect (Navailles and others 2008; David and others 2009)	No effect (Navailles and others 2008; David and others 2009)	↑ (Navailles and others 2008; David and others 2009)
	Antidepressant treatment during adolescence	↑ (Navailles and others 2008)	↑ (Navailles and others 2008)	↑ (Navailles and others 2008)
	Antidepressant treatment during early life stress	No effect (Navailles and others 2008)	No effect (Navailles and others 2008)	No effect (Navailles and others 2008)
	Antidepressant treatment in corticosterone-treated animals	↑↑ (David and others 2009)	↑↑ (David and others 2009)	↑↑ (David and others 2009)

↑ = stimulation; ↑↑ = strong stimulation.

Animal Models of Anxiety-Depression and Adult Hippocampal Neurogenesis

A better understanding of the role of adult neurogenesis in the pathophysiology and the antidepressant-like activity of drugs might come from the use of animal models of anxiety-depression instead of the use of nonstressed animals. Most of the knowledge we have of the pathophysiology of mood disorders comes from the mechanism of action of molecules effective against these disorders. Currently, there are many treatments for depression, including psychotherapy, electroconvulsive therapy, and antidepressant medications. SSRIs are the most commonly prescribed drugs for the treatment of depression, and several anxiety disorders even though their actions at the molecular and cellular levels still remain poorly understood. In addition, only 50% of patients show full remission following treatment with SSRIs, although up to 80% shows partial responses (Nestler and others 2002). Like the first-generation antidepressants, SSRIs require at least 4 to 6 weeks before achieving therapeutic benefits (Wong and Licinio 2001). The paradox between the rapid increase in serotonin levels in vivo and the delayed onset of antidepressant action has led us to postulate that acute enhancement of serotonin transmission alone is not sufficient for the therapeutic effects of SSRIs, but structural or functional changes that take place over time may be required.

Studies involving the observation of depressed patients have given rise to various hypotheses concerning the etiology of depression, and some of these hypotheses have given rise to corresponding animal models. Many animal models, which have been used for the selection of putative antidepressants, have been based on the simple amine deficiency theory, which postulates that depression arises as a result of a deficiency in biogenic amine neurotransmitters in the synaptic cleft.

To address these caveats, several animal behavioral paradigms that respond to chronic but not subchronic antidepressant treatment have been developed. These include the novelty-suppressed feeding test, the novelty-induced hypophagia test, the chronic mild stress/chronic unpredictable stress test (CMS/UCMS), and the social defeat test (Berton and others 2006; Dulawa and others

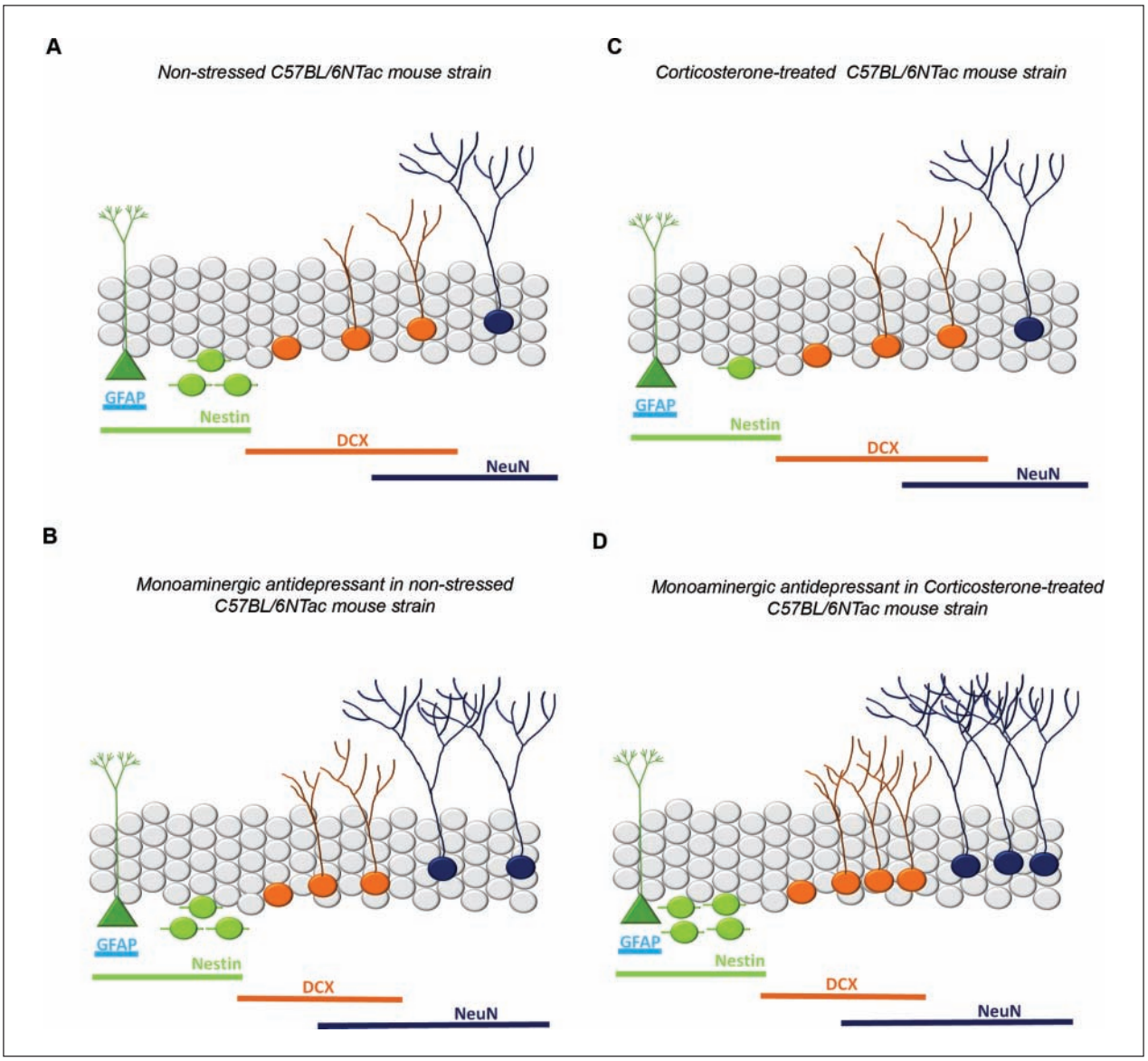

Figure 4. Chronic fluoxetine stimulates dendritic maturation and synaptic plasticity of newborn granule cells in C57BL/6NTac, a possible mechanism for antidepressant action only in corticosterone-treated animals. (A, B) From left to right, anatomical and functional stages during neuronal differentiation and maturation, including quiescent, positive radial glia-like progenitors (green), rapidly amplifying neural progenitors (light green), immature granule cells (orange), and mature granule cells (blue). Bottom panels show immunohistochemical markers for each stage. Strain differences in hippocampal adult proliferation have been reported (Schauwecker 2006; Navailles and others 2008), and C57BL/6 strain exhibits one of the highest numbers of proliferating cells within the subgranular zone, as compared to those of other strains of mice (A). Fluoxetine in C57BL/6NTac strain, contrary to 129SvEv mice, only affects the maturation step (B). (C, D) Chronic corticosterone exposure mimicked the effect of chronic stress on cell proliferation, decreasing the number of BrdU-positive cells in the dentate gyrus of the adult mouse hippocampus (C). The effects of corticosterone on neurogenesis are limited to the proliferation stage and not the survival or maturation of newborn neurons. Interestingly, the effects of fluoxetine on all stages of neurogenesis (proliferation, differentiation, and survival) were more pronounced in corticosterone-treated mice than in controls. It is possible that our model of corticosterone-induced stress may increase the dynamic range in which fluoxetine exerts its effects on different stages of neurogenesis in the adult hippocampus (D).

2004; Santarelli and others 2003). Together, these tests have been applied to the study of the molecular neurobiology of depression, in particular, through testing the basal behavioral changes to antidepressants in genetically manipulated mice. Unfortunately, environmental stress manipulations in rodents, such as the CMS, are hampered

Table 2. Implications of Functional Integration of Adult-Born Hippocampal Neurons in Antidepressant-Like Activity of Monoaminergic Antidepressant

Conditions	Species	Strain	Animal Model	Method of Ablation	Monoaminergic Antidepressant	Effects
No stress	Mouse	129SvEv	NSF	Low dose of X-ray	Fluoxetine, imipramine	Neurogenesis dependent (Santarelli and others 2003; Wang and others 2008)
	Mouse	BALB/cJ	FST, NIH	Low dose of X-ray	Fluoxetine	Neurogenesis independent (Holick and others 2008)
Chronic mild stress	Rat	Fisher	FST, OF	X-ray	Fluoxetine	Neurogenesis dependent (FST)/ independent (OF) (Airan and others 2007)
Chronic mild stress	Rat	Wistar	SC, FST, NSF	Antimitotic agent MAM	Fluoxetine	Neurogenesis dependent (NSF)/ independent (SC, FST) (Bessa and others 2009)
Unpredictable chronic mild stress	Mouse	BALB/cJ	CS, ST, NSF, A	Low dose of X-ray	Fluoxetine, imipramine	Neurogenesis dependent (CS, ST, NSF)/independent (A) (Surget and others 2008)
Chronic corticosterone administration	Mouse	C57/Bl6NTac	NSF, OF, FST	Low dose of X-ray	Fluoxetine	Neurogenesis dependent (NSF)/ independent (OF, FST) (David and others 2009)

FST = forced swim test; OF = open field; NIH = novelty-induced hypophagia; SC = sucrose consumption; NSF = novelty suppressed-feeding; CS = coat state; ST = splash test; A = actimeter.

by protocol variability and reported difficulties in replication, highlighting the need for a depression model easily replicable between laboratories (see Gourley and Taylor 2009 for review). We decided to supply mice with exogenous corticosterone, and then, we analyzed the behavioral consequences. Corticosterone is a hormone produced by the adrenal gland in response to stress and is found to be elevated in several commonly used animal models of depression including restraint stress, psychosocial stress, forced swimming, and exposure to predator odor. There is also evidence from human studies that depression is often associated with dysfunctions of the hypothalamic-pituitary-adrenal (HPA) axis (Holsboer 2008). Furthermore, the use of exogenously administered corticosterone has validity as a model to study chronic stress and depression (Gourley and others 2008). Thus, we and other groups (David and others 2009; Ardayfio and Kim 2006; Murray and others 2008; Gourley and others 2008) utilized chronic corticosterone treatment to develop and refine a mouse model displaying hallmark characteristics of anxiety and depression. Chronic treatment with low amounts (35 mg/mL delivered in the drinking water) of the glucocorticoid corticosterone (CORT) was sufficient to induce anxiety/depression as measured by several appropriate behavioral tests. Importantly, in this mouse model, antidepressants are effective only in the corticosterone-treated animals. Thus, the "corticosterone model" mimics observations made in humans in the sense that antidepressants are generally thought to have no major effects in patients who are not clinically depressed. We have used the "CORT model" to investigate the role of adult hippocampal neurogenesis in the anxiolytic/antidepressant-like action of fluoxetine (David and others 2009).

Antidepressants Elicit Neurogenesis-Dependent and -Independent Effects

In their recent paper, Boldrini and others (2009) suggested that future studies in humans must determine what degree of antidepressant response is linked to increased neurogenesis in adult hippocampus. So far, preclinical studies in rodents using loss-of-function approaches that selectively abolish adult hippocampal neurogenesis have been used to study the relationship between network activity and dentate gyrus neurogenesis and their contributions to the behavioral effects of antidepressants in nonstressed and stressed animals (Table 2).

Antidepressants Elicit Neurogenesis-Dependent and -Independent Effects in Nonstressed Animals

Questions have also been raised regarding the proposal by Santarelli and others (2003) that hippocampal neurogenesis is essential for the manifestation of behavioral improvement after the administration of antidepressants. Since this first study in nonstressed rodents, others have

shown that the behavioral effects of monoaminergic antidepressants are dependent upon the presence of hippocampal neurogenesis (Wang and others 2008). However, in one strain of mice (BALB/cJ), X-rays did not block the response to antidepressants in various tests such as the forced swim test and the novelty-induced hypophagia test (Holick and others 2008). Noteworthy in this latest study and in a recent report, chronic fluoxetine treatment also failed to increase hippocampal neurogenesis (Holick and others 2008; Huang and others 2008). More generally, depending on the experimental conditions and the treatment, the behavioral effects of drugs with anxiolytic/antidepressant-like activities might be mediated via neurogenesis-dependent or -independent pathways. Indeed, beneficial effects of environmental enrichment and exercise on learning and on anxiety-like behavior can occur independently of increased adult hippocampal neurogenesis in mice (Meshi and others 2006). Furthermore, the anxiolytic and antidepressant-like effects of a melanin-concentrating hormone receptor antagonist do not require neurogenesis (David and others 2007). However, X-ray of the hippocampus blocked both the neurogenic and anxiolytic- and antidepressant-like effects of chronic HU210 (potent synthetic cannabinoid) treatment, suggesting that chronic HU210 treatment likely acts via promotion of hippocampal neurogenesis (Jiang and others 2005). Thus, in nonstressed conditions, antidepressants are likely to exert their behavioral effects through neurogenesis-dependent and neurogenesis-independent pathways.

To examine whether antidepressant-induced neurogenesis may simply be an epiphenomenon, the therapeutic efficacy of the antidepressants in subsets of animal models of anxiety/depression, in which a decrease or an ablation of neurogenesis has been done, might be an alternative. In the "CORT model," in some of the neurogenesis readouts in the paper (proliferation and maturation), fluoxetine is much more effective in corticosterone-treated animals, suggesting that a model of stress may increase the dynamic range in which fluoxetine can exert its effects on specific steps of neurogenesis.

Antidepressants Elicit Neurogenesis-Dependent and -Independent Effects in Stressed Animals

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 rats and specifically the role of neurogenesis in depression-relevant neurophysiology and behavior (Airan and others 2007). Using irradiation to ablate neurogenesis, Airan and others (2007) also found that antidepressant behavioral efficacy in the forced swim test in rats 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 rats 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 (Bessa and others 2009). Indeed, using the 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 antidepressant drugs studied (imipramine, fluoxetine) reduced the hyperanxious state observed in CMS-exposed rats, even though neurogenesis was blocked. Overall, authors concluded that antidepressants reestablished neuronal plasticity in the 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. Previously, Surget and others (2008) presented important evidence for both neurogenesis-dependent and -independent mechanisms for the reversal of stress-induced behaviors by antidepressant drugs, including fluoxetine. We think that our paper, using a different model of stress, brings a mechanistic approach to further elucidate the neurogenesis-independent pathways. Indeed, we are showing that one potential neurogenesis-independent mechanism mediating the effects of SSRIs may be the β-arrestin signaling pathway.

Concluding Remarks

The idea of fluoxetine, or more general monoaminergic antidepressants, eliciting effects via distinct mechanisms raises the question whether the same reasoning applies to the therapeutic effects of all antidepressant drugs. For example, might antidepressants work through a confluence of effects on both cognitive functions and mood? In the brain, would this translate to a distinction between hippocampal and other limbic structure–dependent effects of antidepressants? In accordance with this hypothesis, it has been suggested that the effects of antidepressants on mood may be neurogenesis independent, while those on anxiety may be neurogenesis dependent (Airan and others

2007; Bessa and others 2009; Surget and others 2008; David and others 2009). This is an important question as developers of future drugs need to determine whether compounds that directly stimulate neurogenesis would be effective as antidepressants or would only ameliorate cognitive deficits.

Authors' Note

Denis J. David and Jingwen Wang contributed equally to this work.

Declaration of Conflicting Interests

The authors declared a potential conflict of interest (e.g., a financial relationship with the commercial organizations or products discussed in this article) as follows: Rene Hen receives compensation as a consultant for Braincells Inc. and AstraZeneca in relation to the generation of novel antidepressants.

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