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Increasing Hippocampal Neurogenesis: A Novel Mechanism for Antidepressant Drugs

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Abstract: The birth of new neurons, or neurogenesis, in the hippocampal formation has been demonstrated throughout the lifetime of multiple species including humans. A major finding in the field of depression is that treatment with antidepressant drugs increases hippocampal neurogenesis. This review presents a current summary of this field of study and presents the hypothesis that increasing adult hippocampal neurogenesis may be a new drug target or mechanism for future antidepressant drugs. It has been demonstrated that multiple classes of antidepressant drugs increase hippocampal cell proliferation and neurogenesis in a chronic and not acute time course, which corresponds to the therapeutic time course necessary for effects. Conversely, animal models of depression or stress paradigms decrease cell proliferation. Clinically, there is evidence of reduced hippocampal volume in patients with major depressive disorder or other affective disorders. Taken together, this data indicates that reduced hippocampal cell number may be involved in the pathophysiology of depression and reversal of this may be one way the antidepressant drugs exert their effects. We hypothesize that the next generation of antidepressant drugs will, in addition to their effects on known transmitter or second messenger systems, involve either direct or indirect targeting of neurogenic factors. In addition, the ability of novel compounds to be tested for the neurogenic potential may become an additional way to evaluate a compound for putative antidepressant effects.

Key Words: Neurogenesis; depression; proliferation; hippocampus; serotonin.

INTRODUCTION

Depression affects millions of people per year, with a lifetime prevalence of 16% [1]. Although antidepressant drugs are an immense market, representing over $13 billion per year, there are still multiple unmet needs in treatment for depression. These needs include improved efficacy (20-30% of patients are resistant to current drug therapies)[2], reduced sexual dysfunction and decreasing the current 2-3 week lag to onset to therapeutic efficacy. The search for new antidepressant drugs that address these needs, as well as the search for new mechanisms and understanding of antidepressant action, are the current driving forces in antidepressant drug discovery.

A major finding in the field of depression in the last few years is that antidepressant drugs increase the number of new neurons born in the hippocampus of adult animals [3]. The idea that the adult brain has a finite number of cells dates back to the early work of Ramon y Cajal, who noted that in contrast to a developing brain, the adult brain has no capacity for regeneration [4]. The idea that brain cells are generated throughout the lifetime of animals and humans is relatively recent, but is now a large and increasing field of study. This discovery has had a large impact, and caused a paradigm shift, on the understanding of the mechanisms of antidepressant action and the pathophysiology of depression. We hypothesize that in addition to antidepressants' affect on monoamine and second messenger systems, the next generation of antidepressants may also produce their therapeutic effects by increasing hippocampal neurogenesis or by otherwise affecting brain morphology. We will review the existing literature that has brought us to this hypothesis, and suggest that increased adult cell proliferation and neurogenesis could be both a target for, as well as a way to evaluate, novel antidepressant compounds.

In the adult rodent, neurogenesis has been reported in three main areas of the brain: 1) The olfactory bulb; 2) The subventricular zone of the lateral ventricle, which is the cell layer adjacent to the lateral ventricle in the striatum, whose cells migrate via the rostral migratory stream to the olfactory bulb; 3) The subgranular zone (SGZ) of the dentate gyrus of the hippocampus [5]. The SGZ comprises the border between the granule cell layer and the hilus. Hippocampal cell proliferation, in contrast to the proliferation in other neurogenic areas in the brain, will be the major focus of this review.

METHODOLOGIES TO STUDY NEUROGENESIS

The first studies of hippocampal neurogenesis utilized [^{3}H]-thymidine, which could be visualized using autoradiography [6, 7]. Recent studies primarily use the non-radioactive thymidine analog 5'bromo-2-deoxyuridine (BrdU) as a marker for dividing cells. BrdU is injected intraperitoneally into the animal, crosses the blood brain barrier and is taken up into the DNA of cells during s-phase of mitosis [8, 9]. The animal is sacrificed at different time points after the BrdU injection, and subsequently the brains are sliced and stained for BrdU-positive cells using immunohistochemistry. A BrdU-positive cell is considered to be a newly-born cell,

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and the numbers of BrdU-positive cells are quantified using a modified stereology protocol [3].

In addition to determining the number of BrdU-positive cells, the phenotype of the cells can also be determined, using double or triple immunohistochemistry. If the animals are sacrificed at short time points after BrdU injection, the cells can express immature neuronal markers such as doublecortin [10], TUJ1, TUC-4 [11] or the immature glial marker NG2 [12]. If the animals are allowed to survive for longer time points, antibodies for cell migration (PSA-NCAM) [13], mature neurons (NeuN, Calbindin) [3, 14] and mature glia (S100B, GFAP) [3, 15] are used to determine the final fate of the BrdU-positive cells. Double- and triple-labeling experiments have demonstrated that the cells born in the SGZ and hilus mature into neurons and glia and integrate into the granule cell layer [16]. Using retroviruses that label cells that can be identified in slice preparations, electrophysiology experiments have demonstrated that these cells are functional and send out correct synaptic connections [17].

One of the main problems with studying hippocampal cell proliferation and neurogenesis is the methodology used to quantitate the number of newly-born cells. The most stringent and validated technique is the optical fractionator method, which eliminates many of the inherent biases encountered in cell counting and analysis [18]. However, this requires a microscope with a z-plane stage encoder and software, which can be prohibitively expensive for any laboratory that does not intend to utilize the equipment full-time to study neurogenesis.

Most laboratories utilize a semi-quantitative manual counting method to determine the number of BrdU-positive cells [3]. After fixation and preservation, the brain is cut in serial sections. Every nth section (usually 8-10) throughout the hippocampus or other brain region of interest is processed for BrdU immunohistochemistry. In each processed slice, all of the BrdU-positive cells in the region of interest, for example the SGZ and granule cell layer, is counted manually by a researcher blinded to the study conditions. In order to successfully utilize this ”object counting” technique, the following requirements must be met: that no BrdU-labeled cells be counted twice and that the area counted be consistent in each section and brain region.

The main issue in the quantitation is the time involved in counting the individual cells; all of the cells need to be counted at 400x or 1000x in order to get an accurate count of the BrdU-positive cell number [19]. Since there is no easy or automated assay, this time-intensive protocol limits the ability of a single researcher to quickly evaluate drugs or screen for compounds that increase cell proliferation and neurogenesis. However, there are a number of reasons for the necessity of a non-automated system. Unlike radioactive thymidine, where the number of grains per cell can be quantified, BrdU uptake into cells is non-quantitative, so that there is no constant size of labeled cells or density of consistent cell labeling. BrdU-positive cells also have varied morphology or are in clusters, so there is no size exclusion principle one could use for automated cell counting [19]. Automated technologies are currently being investigated. Once they have been implemented, the ability to do multiple experiments will be of immeasurable value to the field.

The time points at which the animals are sacrificed after the BrdU injection will determine the type of study and the information that can be acquired [20]. Studies are usually delineated as ‘proliferation’ or ‘neurogenesis’ studies. Often these words are used interchangeably and incorrectly. In a strict sense of the definition, “proliferation” means one round of cell division. Cameron and McKay have elegantly demonstrated that in the adult rat, the s-phase of cell division is approximately 2 hours [11]. Since BrdU is incorporated into the DNA of cells during the s-phase, sacrificing an animal 2 hours after a single BrdU injection will provide the cell counts of one round of cell division or proliferation. However, among different laboratories, the word ‘proliferation’ is used to mean any short timepoint after the BrdU injection. When animals are sacrificed at different timepoints after BrdU injection, the number of rounds of cell division that have taken place will differ. This can complicate comparison and interpretation of results among studies.

‘Neurogenesis’ indicates a study where the animals are allowed to survive long enough after the BrdU injection for the newly-born BrdU-positive cells to express their mature phenotype. In the hippocampus, although a small percentage of the BrdU-positive cells do turn into glia, the majority become neurons, thereby predominantly producing neurogenesis. The reader is cautioned to keep in mind the differences between proliferation and neurogenesis experiments. In addition, a drug or behavioral treatment that affects proliferation does not necessarily also affect neurogenesis, and care must be taken in interpretation of experiments where only one experimental paradigm, either proliferation or neurogenesis was utilized.

The majority of experiments using BrdU look at proliferation or neurogenesis. These studies are constructed so that the animal is given a drug or behavioral treatment, after which the BrdU is injected and the animals sacrificed. In this case, the number of BrdU-positive cells is due to the effect of the experimental manipulation on stem cell proliferation. However, another type of paradigm is to look at the effect of an experimental manipulation or drug on cell survival. BrdU can be injected first (under normal cell division conditions) and then the animal is subjected to the experimental paradigm. In this instance, the number of BrdU-positive cells counted when the animal is sacrificed would be due to the effect of the paradigm on cells maturation and survival. It is imperative to understand the differences in injection and sacrifice time in order to make meaningful comparisons between studies. This is especially important when trying to replicate or extend the studies conducted by other laboratories.

BACKGROUND OF STUDIES INVESTIGATING ADULT HIPPOCAMPAL NEUROGENESIS

The first reports of adult hippocampal neurogenesis in the adult rat occurred in 1965 with Altman and Das [7], followed by Kaplan and Hinds [6] in 1977. These investigators injected adult animals with [^{3}H]-thymidine and reported that the [^{3}H]-thymidine-positive cells matured into a neuronal phenotype, indicating that neurogenesis occurred in the adult brain. However, these findings were ignored and in fact denigrated for a number of years (for a full discussion of

this, see Kaplan, "Environment complexity stimulated visual cortex neurogenesis: death of a dogma and a research career" [21]). Olfactory bulb neurogenesis, in contrast, had been reported and accepted by the scientific community for some time before this and is still a prolific field of study [22]. However, at the time of the above-mentioned studies, functional sensory olfactory bulb neurogenesis was considered to be a separate entity from hippocampal or cortical neurogenesis.

In 1983, Goldman and Nottebaum demonstrated adult neurogenesis in the songbird [23]. This was found in the areas used in song acquisition and learning, which argued for a functional component of neurogenesis in a cortical area. However, the idea that neurogenesis occurred in mammals was still not accepted until it was demonstrated by McEwan and Gould [24] that in the adult rat, cells in the SGZ of the dentate gyrus can proliferate de novo and mature into neurons. Gould *et al*. also reported that administration of adrenal steroids decreased hippocampal neurogenesis in the rat [25] and followed this with reports that physical or psychosocial stress decreased cell proliferation and neurogenesis in the tree shrew and marmoset [26, 27]. Subsequently Gage and colleagues [28] demonstrated that mice placed in an enriched environment had increased neurogenesis, indicating that in the rodent, positive modulation of neurogenesis could occur due to environmental stimulation.

In 1999, Gould and colleagues reported that hippocampal neurogenesis could be seen in adult macaque monkeys [29], while Eriksson and Gage [30] determined that hippocampal neurogenesis occurred in the human, using post-mortem brains of cancer patients who had been injected with BrdU. Double labeling of BrdU and mature neuronal markers in the granule cell layer validated adult human hippocampal neurogenesis. Gould *et al*. further reported that neurogenesis could be observed in the prefrontal, inferior temporal and posterior parietal cortex of adult primates [31, 32]; however, cortical neurogenesis is still questioned and debated in the scientific community [33].

The studies described above have given birth to an entirely new field, the study of the regulation and function of adult neurogenesis. There are a large number of variables that have been shown to either positively or negatively affect neurogenesis such as species, strain, age, gender, hormone level, exercise, learning, drug treatment and behavioral paradigms (for review, see [34]).

Among the variables regulating proliferation and neurogenesis is stress and antidepressant treatment. Stress paradigms have been shown to decrease neurogenesis, while antidepressant drugs have the opposite effects, to increase proliferation and neurogenesis [35]. These findings have had a large impact on the field of depression. Both clinical and preclinical evidence have implicated changes in neurogenesis in depressive disorders or models of depression, and a current hypothesis that is being investigated by many labs [35, 50] is that antidepressants may exert their effects by increasing hippocampal neurogenesis.

STRESS AND NEUROGENESIS

Researchers studying depression realized that adult hippocampal neurogenesis had implications for their work based upon the studies that demonstrated that stress and glucocorticoids induced a decrease in the number of proliferating cells in the SGZ. The link comes from the many hypothesized clinical and preclinical interactions between stress and depression [35]. In addition, many animal models and theories of depression are stress-based and most animal models of depression include a stress (either physical or psychosocial stress) component.

In some of the first work implicating a connection between stress and neurogenesis, Gould *et al*. demonstrated that a one hour exposure to a psychosocial stress paradigm, the resident-intruder paradigm, reduced cell proliferation in both tree shrew and marmosets [26, 27]. In rats, exposure to fox odor, which is a stressor, also decreases proliferation as measured by either BrdU or [^{3}H]-thymidine labeling [36, 37]. It has been demonstrated that footshock produces an immediate decrease in proliferation, and repeated inescapable footshock produces a decrease in proliferation that is still detected 7 days after the shock [15]. Repeated restraint stress also produces a decrease in cell proliferation [38].

These acute decreases in hippocampal cell proliferation are hypothesized to be due to a stress-induced release of glucocorticoids [39]. Administration of exogenous corticosteroids produces a decrease in cell proliferation and neurogenesis [25], whereas adrenalectomy prevents the stress-induced decrease in proliferation [37]. Although glucocorticoids are implicated in the stress-induced decrease of proliferation, the newly-born cells in the SGZ do not express either Type1 or Type II glucocorticoid receptors [40]. Therefore, glucocorticoids downregulate stem cell proliferation by acting indirectly on the newborn cells. It may be that the glucocorticoids directly affect the local environment of the SGZ to negatively affect stem cell proliferation.

It has been demonstrated, however, that a decrease in cell proliferation does not require a constant elevation of glucocorticoid levels. Seven days after exposure to inescapable footshock, a decrease in hippocampal cell proliferation is seen, even though the rats have normal serum corticosterone (CORT) levels at this time point [15]. In this case, we hypothesize that the acute stress (footshock) has activated regulatory pathways to produce a long-lasting suppression of proliferation after the stress-induced CORT increase has subsided. Given that hippocampal cell proliferation has been implicated in learning paradigms [41], cognitive impairments under times of acute stress [42] may be related to this suppression of cell proliferation.

The effect of stress on cell survival is less clear. In a study designed to investigate the effect of stress on cells already born in the SGZ, rats were injected with BrdU on Day 1 and subjected to 2 hours of restraint stress on Days 4 through 9 or on Days 10 through 17, with all rats sacrificed on Day 28. Neither of the stress paradigms produced a decrease in the number of BrdU-positive cells at the 28 day time point, indicating that glucocorticoid release did not decrease survival (Malberg and Duman, unpublished data). However, other investigators have demonstrated that longer exposure to stress such as 3 or 6 weeks of restraint stress [38], or 18 days of psychosocial stress [43], decreases cell survival. It may be that the amount of stress needed to affect proliferation may be much less than is needed to affect

survival. In chronically depressed humans, both proliferation and survival may be affected, which may lead to the reduced hippocampal volume seen in depressive disorders [44].

Previous investigators have demonstrated that chronic stress produces atrophy and dendritic arborization of CA3 pyramidal neurons [45]. This dendritic remodeling is hypothesized to be due to the stress-induced release of excitatory amino acids and corticosteroid activation [46], and can be reversed by the atypical antidepressant tianeptine [47]. Collectively, stress has been shown in many experiments to have negative effects on hippocampal organization and synaptic plasticity [46]. We can hypothesize that stress may be causing three separate deleterious effects to cells, by affecting proliferation, survival and dendritic remodeling. This "triple threat" may lead to a depressive phenotype or depressive symptoms.

The multitude of factors that convert physical or psychosocial stress into a cellular response is unknown. Recently, the transcription factor nuclear factor kappaB (NF-kappaB) has been identified as a potential biological regulator that converts psychosocial stress into mononuclear cellular activation. Bierhaus *et al*. [48] hypothesize that activation of NFKB would trigger the cellular pathways for the neuroendocrine responses to stressful psychosocial events. This type of study will become very common in the future, since the search to find novel antidepressants will most likely center on identification of the factors that are affected by or mediate the stress response.

ANTIDEPRESSANTS AND NEUROGENESIS

Given the interaction between stress, depression, and adult hippocampal neurogenesis, the question of whether antidepressants had an effect on adult neurogenesis was investigated. Accordingly, it was reported that multiple classes of antidepressant drugs as well as electroconvulsive shock (ECS) produced an increase in cell proliferation and neurogenesis in the adult rat hippocampus [3, 49]. Fluoxetine (specific serotonin reuptake inhibitor), tranylcypromine (monoamine oxidase inhibitor), reboxetine (specific norepinephrine reuptake inhibitor), imipramine (tricyclic) [50] and rolipram (phosphodiesterase-IV inhibitor hypothesized to be an antidepressant) [51] have all been shown to increase cell proliferation and neurogenesis. Chronic lithium treatment was also seen to increase neurogenesis [52]. Compared to the chemical antidepressants, ECS induces the greatest amount of cell proliferation [3]. ECS is the most clinically effective treatment for drug-resistant depression [53], although it is unknown if that is due to its strong proliferative effect.

It has been demonstrated by various investigators that a chronic dosing paradigm is needed to see a significant increase in cell proliferation. Fluoxetine treatment did not increase cell proliferation after 1 or 5 days of treatment, but did increase proliferation following 14 days of treatment [3]. Santarelli *et al*. [50] has also reported that an increase in cell proliferation was seen after 11 but not 5 days of fluoxetine. Analogously, a single dose of rolipram was not effective to increase proliferation but 21 days of rolipram treatment increased cell proliferation [51]. Although a single ECS treatment was effective in increasing cell proliferation, it has been shown that a greater number of ECS treatments produce greater increases in proliferation [49]. Clinically, patients receive multiple, not single, ECS sessions [53]. It is not known if the increased cell proliferation from the multiple sessions translates into clinical efficacy.

Following these studies, further investigations examined the question of duration of drug treatment on cell proliferation and cell survival. There was no additional increase in cell proliferation when animals were given fluoxetine for either 14 or 28 days. This indicates that after 14 days of fluoxetine treatment, a plateau in the increased rate of proliferation was reached [3]. This effect was also reported by Santarelli [50], who saw no further increase in proliferation between 11 and 28 days of fluoxetine administration. In contrast to these results, it was found that a longer amount of treatment time was required for fluoxetine to increase cell survival. In a study designed to determine the effect of duration of treatment on cell survival, animals were given an injection of BrdU followed by either 14 or 28 days of fluoxetine and sacrificed on Day 28 [13]. It was shown that 28 but not 14 days of fluoxetine was necessary to increase the total number of cells, which in this study represented the effect of antidepressants on cell survival. Thus, a longer period of antidepressant administration is needed to increase cell survival compared to proliferation. These different time courses may represent different pathways for proliferation and survival, with a longer time course needed for activation of survival pathways.

Following these investigations, it was of interest to determine the ultimate differentiation of the proliferating stem cells. In antidepressant- and ECS-treated animals, the majority of the BrdU-positive cells became neurons and not glia, as identified by triple labeling and confocal microscopy [3, 13]. This indicates that antidepressant treatment produces a net number of new neurons (neurogenesis). The percentage of BrdU-positive cells that became neurons or glia was the same between antidepressant, ECS and control-treated animals, indicating that the antidepressants do not have an influence on the differentiation of cells into their phenotypes.

Further studies demonstrated that an antidepressant-induced increase in neurogenesis is restricted to the SGZ of the hippocampus, with no affect on subventricular zone neurogenesis [3]. Additionally, the ability to increase proliferation seems to be restricted to the antidepressant drugs, since antipsychotic drugs such as haloperidol and clozapine do not produce changes in hippocampal proliferation [3, 19]. Other psychotropic drugs such as morphine and heroin produce decreases in proliferation and neurogenesis [54] and cocaine has no effect [19].

Taken together, these studies demonstrate that antidepressants specifically increase hippocampal cell proliferation and neurogenesis. Based on the fact that multiple drug classes and ECS increase proliferation and neurogenesis, this increase may represent a novel mechanism or pathway from which the new generation of antidepressants will be discovered. A working hypothesis in many laboratories is that a factor or factors within neurogenic pathways may represent a novel target for antidepressant drugs.

In addition to investigating neurogenic pathways for new targets, another hypothesis from a drug screening perspective

is that increased neurogenesis may be used as a marker to test unknown compounds for antidepressant potential. In addition to behavioral testing, it may that a drugs' ability to increase neurogenesis may be preclinical evidence that it will be an effective antidepressant. Due to the time-consuming task of analyzing and counting the cells, the number of novel compounds that can be analyzed for increases in proliferation and neurogenesis are severely limited. However, at least one paper to date has been published detailing a putative antidepressant based on its ability to increase neurogenesis [55]. The further development of a drug that specifically modulates neurogenic activity without other pharmacological effects that demonstrates clinical efficacy would provide proof of concept in the neurogenesis hypothesis of antidepressant mechanisms.

In addition to having a novel way to evaluate antidepressant activity, the time course of administration needed to increase cell proliferation may provide information about the time course needed to produce therapeutic effects. This would be valuable since reduction of the lag between administration and cessation of symptoms is a main objective for the next generation of antidepressant drugs. It has been shown [3, 50, 51] that 2 weeks of antidepressant administration is necessary to induce an increase in cell proliferation and neurogenesis. This is notable because this chronic treatment parallels that needed to demonstrate clinical efficacy. A novel compound that produces an increase in proliferation in a shorter amount of time would be of great interest. One hypothesis is that a shorter treatment time needed to increase hippocampal proliferation and neurogenesis would correspond to a quicker onset clinically, although that has yet to be tested experimentally.

CLINICAL STUDIES

In addition to the abovementioned preclinical evidence, clinical evidence is emerging that supports decreased hippocampal volume in affective disorders as well as evidence that antidepressants reverse and normalize the brain volume after treatment. The first MRI studies demonstrated reduced hippocampal volume in patients with major depressive disorder, PTSD and Cushing's disease [44]. However, it should be noted that alterations in brain morphology are neither limited to the hippocampus nor to just alterations in neurons. Postmortem studies have demonstrated decreases in volume, decreases in glial and neuronal cell number, and reduced cortical thickness in the prefrontal cortex in depressed populations [56]. Current imaging studies have demonstrated reduced size and volume [57] as well as reduced glucose metabolism and blood flow [58] in multiple brain regions in patients with MDD, PTSD or other affective disorders. The majority of the structures associated with the limbic-cortical-striatal-pallidal-thalamic tract have demonstrated structural changes in patients with MDD, PTSD or bipolar disorder [57]. However, many studies looking at similar populations have produced conflicting and even opposite results, and clearly more work is needed in this exciting area of study.

In depression, many but not all studies have found decreased hippocampal volume. When high-resolution MRI was used, depression was associated with significant decreases (8% to 19% decrease) compared to non-depressed controls [57]. Decreased hippocampal volume has been correlated with the amount of time (days) of untreated depression [59, 60].

Although it is impossible to determine if reduced hippocampal volume is the initial insult, a secondary finding induced by another pathological factor, or a marker for depressive disorder, it may be hypothesized that reversal of this decrease could be a target for antidepressant drugs. In support of this, it has been demonstrated that paroxetine treatment increases both cognitive function and hippocampal volume in patients with PTSD [61]. In addition, the correlation between reduced hippocampal volume and duration of the depressive episode dissipates when subjects are taking antidepressants. This indicates that antidepressant administration reverses the depression-induced decrease in hippocampal volume [60]. These clinical studies suggest that the effects of stress and antidepressant treatments in animal models may parallel clinical findings in the human. Further research is needed to delineate this expanding field.

ANIMAL MODELS OF DEPRESSION TO STUDY THE EFFECT OF NEUROGENESIS

One of the challenges to identifying novel antidepressants is the limitation of current animal models of depression. Ideally, one would like the animal models to demonstrate both predictive validity and face validity, with a similar time course of administration to demonstrate both behavioral and therapeutic efficacy. However, most of the current models of depression fall short in one or more of these issues (see Cryan and Lucki [62] for review).

To date, two models of depression have been studied as to their effect on cell proliferation and neurogenesis; learned helplessness in the rat and psychosocial stress in the tree shrew. In both of these models, the stress-induced behavior is reversed by antidepressant treatment. A main advantage of the models is that the antidepressants are tested not in normal animals but in animals that have undergone some sort of physical or psychosocial stress, which is hypothesized to model depression. Both of these models produces effects such as decreased weight and deficits in immunosuppression, which are symptoms seen in clinically depressed patients [63, 64].

In the learned helplessness model of depression, it has been demonstrated that exposure to inescapable shock, which produces a behavioral deficit, also decreases cell proliferation. When rats were treated with for 7 days with fluoxetine after the inescapable shock, which reverses the behavioral deficit, the numbers of BrdU-positive cells were returned to control levels. These data indicate an interaction between behavior, antidepressant action, and proliferation, and shows that the learned helplessness model of depression produces a decrease in proliferation that is reversed by antidepressant treatment [15].

Czeh *et al*. have demonstrated similar effects in the chronic psychosocial stress model using the adult tree shrew. These investigators reported that 7 days of stress exposure in the resident-intruder paradigm produces a decrease in hippocampal volume, cell proliferation rate and N-acetyl-

aspartate, creatine and phosphocreatine concentrations. These stress-induced changes were reversed to normal after 28 days of treatment with the atypical antidepressant tianeptine or the tricyclic antidepressant clomipramine [65, 66]. These data suggest that chronic treatment with antidepressants may overcome the stress-induced reduction of cell proliferation in the dentate gyrus.

In both models, antidepressant treatment normalized or reversed the stress-induced decrease in proliferation; the stressed animals that received antidepressants had the same amount of proliferation as the control animals. This is in contrast to earlier studies, where normal (non-stressed) animals given antidepressants had increased proliferation compared to control animals. This points to the utility of using animal models to test antidepressant drug efficacy, since the effect of drugs on normal and stressed animals may be different. Taken together, these animal models of depression, we demonstrate that antidepressants can reverse the stress-induced decrease in cell proliferation.

One hypothesis is there may be a 'set number' of cells or a setpoint of normal cell proliferation, and when a stressful stimulus decreases the number of cells or rate of proliferation, the organism tries to reverse that decrease. In this case, antidepressant drugs may be activating neurogenic pathways to normalize the cell number, but not to increase it above the setpoint. In indirect support of this are the findings of Sheline [60], who found a correlation between days of untreated depression and reduced hippocampal volume. This correlation disappeared when subjects were taking antidepressants, indicating that the antidepressants reversed the decrease in volume in the depressed subjects. However, there was no positive correlation between lifetime exposure to antidepressants and hippocampal volume. This indicates that continued administration of antidepressants does not increase hippocampal volume above normal and in fact suggests that antidepressant administration normalizes the decrease in hippocampal volume.

In summary, these findings indicate that animal models of depression, which are hypothesized to produce a 'depressive' phenotype, decrease cell proliferation, and this effect can be reversed by antidepressant administration. The search for the neurogenic-suppressing factors which are activated by stressful stimuli or the stress-induced behavior are yet another avenue of continued research. For example, transcriptional profiling between stressed and nonstressed animals is being studied, as well as profiling of animals bred for a helpless or depressed phenotype. A well-controlled profiling study can potentially lead to the identification of novel drug targets.

ABLATION OF PROLIFERATION PREVENTS ANTIDEPRESSANT RESPONSES

Until recently, the majority of studies looking at neurogenesis were correlational studies; animals were given a drug treatment or subjected to a behavioral paradigm that induced changes in proliferation and neurogenesis. However, a number of investigators have been able to prevent cell proliferation or ablate newly-born cells, and these studies have demonstrated a necessity for hippocampal neurogenesis in both learning and antidepressant action. The link between neurogenesis and learning is of interest to the field of depression since it is known that depressed patients also exhibit deficits in cognition [67].

In the first paper that was able to attribute a functional role for the proliferating cells, Shors *et al*. [41] injected rats with a DNA methylating agent that inhibited both central and peripheral cell proliferation. These animals displayed defects in trace conditioning, which is a learning paradigm that requires hippocampal activation. In contrast, delay conditioning, which is a learning paradigm that does not require an intact hippocampus, was unaffected by the cessation in cell proliferation. This demonstrated a necessity for de novo hippocampal cell proliferation in hippocampal-associated learning paradigms. This work has been further extended by Madsen *et al*. [68], who looked at hippocampal-associated tasks at different time points after ablation of cell proliferation by brain x-ray irradiation. In agreement with Shors *et al*., after 8 and 21 days of irradiation, animals performed poorer than controls in a hippocampal-dependent task (place recognition). Notably, these animals showed no decrement in a hippocampal-independent task (object recognition). At longer time points after irradiation, which would parallel the induction of neurogenesis, recovery from the impairment on the hippocampal-associated tasks was observed [41, 68]. These results indicate that these newly born cells serve an important function for learning. However, these same authors have recently published that not all hippocampal-dependent learning is dependent on neurogenesis [69]. This raises the question of neuronal cell types induced by neurogenesis and the specific neuronal circuits involved.

Santarelli *et al*. [50] has provided evidence that suggests that proliferation is necessary for antidepressant drug action. In this study, irradiated animals were given the antidepressants fluoxetine or imipramine and subsequently tested in one of two behavioral paradigms. Importantly, irradiated animals receiving antidepressant treatment were not able to demonstrate the expected behavioral response on either test. These animals were shown to have normal function on a non-hippocampal associated task. This set of data provides the first evidence that hippocampal cell proliferation is necessary for antidepressant action. The authors concluded that the cessation of neuronal proliferation and neurogenesis is responsible for the lack of effect of antidepressants.

Importantly, it should be noted that irradiation methodology has limitations. It has been shown that the newly-born cells are extremely sensitive to x-ray irradiation [70, 71]. In addition to the effect on the proliferating neuronal precursors, the irradiation may have had effects on other factors as well. X-ray irradiation affects differentiation, decreasing the percentage of cells that differentiate into neurons. Irradiation can increase activated microglia, indicating that an inflammatory response is activated. In addition, irradiation disrupts microvascular angiogenesis; the cell clusters that are usually associated with the microvasculature were not seen in irradiated animals [72]. Irradiation may also affect some non-proliferating cells in addition to the neuronal precursors [70]. Furthermore, the x-ray doses used may have affected nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) levels in the cortex and hippocampus;

prenatal exposure to even very low doses of irradiation produce decreases in BDNF and NGF at postnatal day 12 [73]. Although it is difficult to compare studies utilizing young and adult mice, it is possible that the effect of the irradiation on the trophic factors may have contributed to the inability of antidepressants to produce the expected behavioral effect in the irradiated adult mice.

Despite these caveats, this is the first study to demonstrate a causal relationship between neurogenesis and the behavioral effects of antidepressant drugs.

ROLE OF SEROTONIN AND NOREPINEPHRINE IN PROLIFERATION AND NEUROGENESIS

The majority of chemical antidepressants, especially those that have been shown to increase proliferation and neurogenesis, act on either the serotonin and/or norepinephrine systems. Accordingly, the role of these neurotransmitters and their specific receptors, in both baseline and antidepressant-induced neurogenesis is an intensive area of investigation.

Brezun and Daszuta [74-76] have demonstrated in a number of studies that serotonergic activation is necessary for hippocampal cell proliferation. Lesions of the dorsal and medial raphe nuclei with the serotonergic neurotoxin 5, 7-dihydroxytryptamine produces long-term decreases in the number of newly generated granule cells identified with BrdU. This decrease in proliferation can be reversed with exogenous raphe graphs, and recovery from the lesions via reinnervation also reverses this effect. This demonstrates that an intact serotonin system is necessary for normal levels of proliferation and neurogenesis.

The role of specific serotonin (5HT) receptors in mediating neurogenesis *in vivo* has not been extensively studied, with the exception of the $5HT_{1A}$ receptor, which has been targeted using both pharmacological tools as well as knockout mice. Both chronic (28-day) and acute treatment with 8-OHDPAT, the prototypical $5HT_{1A}$ agonist, increases cell proliferation in the adult mouse hippocampus [50, 77]. Conversely, acute administration of WAY-100635, a selective $5HT_{1A}$ antagonist produces an immediate decrease in proliferation [78]. However, we have demonstrated that chronic (14-day) administration of another $5HT_{1A}$ antagonist, WAY-405, when administered in combination with fluoxetine, does not alter fluoxetine's neurogenic effects. We report that the combination of the two drugs (WAY-405 + fluoxetine) increased cell proliferation to the same extent as fluoxetine alone (unpublished data).

Santarelli *et al*. has suggested that 5HT1A receptors are necessary for the behavioral and neurogenic effects of fluoxetine [50]. In a recent study, fluoxetine-treated $5HT_{1A}$ knockout mice do not show either the expected behavioral changes or the expected increase in cell proliferation. However, imipramine treatment produced the expected behavior and increased hippocampal cell proliferation in the $5HT_{1A}$ knockout mice. These authors concluded that $5HT_{1A}$ receptor stimulation may be necessary for the behavioral and neurogenic effects of fluoxetine but are not necessary for the actions of other antidepressants. However, since the role of the $5HT_{1A}$ receptor in the $5HT_{1A}$ knockout mice contrast with that of known selective $5HT_{1A}$ antagonists, it is possible that the results reflect the role of $5HT_{1A}$ receptor in development. In addition, the $5HT_{1A}$ partial agonist buspirone [79] has not been shown to have antidepressant efficacy in the clinic when administered alone, but there are suggestions that in combination with an SSRI there is enhanced onset and efficacy. Furthermore the $5HT_{1A}$ agonist 8-OH-DPAT decreases cell proliferation. Clearly, the role of $5HT_{1A}$ receptors in antidepressant-associated proliferation and neurogenesis needs further clarification.

Studies are underway to determine the role of norephinephrine in neurogenesis, since inhibition of the norepinephrine transporter is a target of many antidepressant drugs used in the clinic. Trycyclic antidepressants as well as the specific noradrenergic uptake inhibitor reboxetine increase hippocampal cell proliferation and neurogenesis in the adult rat and mouse [3, 51]. Conversely, full-brain depletion of norepinephrine using a specific noradrenergic neurotoxin decreases the proliferation of cells in the adult rat hippocampus [80]. However, noradrenergic depletion does not affect survival of cells that have already been born; if BrdU is injected before norepinephrine depletion, there is no change on the final numbers of BrdU-positive cells. Therefore, norepinephrine depletion seems to affect proliferation but not survival or maturation. Laifenfeld *et al*. [81] have shown using transcriptional profiling that treating PC12 cells with norepinephrine induces the upregulation of genes that are involved in synaptic plasticity. Taken together, norepinephrine may have both direct proliferative effects on cell proliferation, and indirect effects on survival by activating genes that in turn activate other proliferative, neurogenic or survival pathways. Theoretically, antidepressants could affect a number of steps in any of the pathways.

TROPHIC FACTORS AS MEDIATORS OF NEUROGENESIS

In addition to modulating serotonin and norepinephrine levels and accordingly, stimulation of multiple receptors, another common effect of different antidepressant drugs is their activation of trophic factors. Multiple classes of antidepressants increase hippocampal BDNF mRNA and protein [82], and increased BDNF levels have been observed in patients taking antidepressants [83]. Chronic stress in rats downregulates BDNF in the dentate gyrus of the hippocampus, this effect is reversed by chronic antidepressant treatment [82]. BDNF administered directly into the lateral ventricle or hippocampus has been shown to possess antidepressant properties in both the forced swim test and learned helplessness paradigms [84, 85]. BDNF has also been shown to be neurogenic when injected into the brain [86] and in fact induces cell proliferation and neurogenesis in areas where these phenomenon are not usually observed [87]. BDNF is activated by the cyclic-AMP (cAMP) response element-binding protein (CREB), and the cAMP-CREB-BDNF pathway is a large field of study in the antidepressant literature (see D'Sa and Duman [88] for review).

CREB is implicated in neural plasticity, antidepressant action and cell proliferation. It is upregulated in the hippocampus after chronic antidepressant treatment, indicating that upregulating CREB is one mechanism by which antide-

pressants may exert their effects [89]. Rolipram, a phosphodiesterase -IV inhibitor that activates the cAMP cascade and is hypothesized to be an antidepressant, has been shown to increase proliferation and neurogenesis in a chronic time-course [90]. Activated or phosphorylated CREB has been identified in the newly-born neurons in the SGZ, as seen by double labeling with BrdU [13]. Conversely, cell proliferation is decreased in transgenic mice expressing a dominant negative mutant of CREB [51].

However, it should be noted that CREB may not be necessary for all actions of antidepressant drugs. Conti *et al*. [91] have reported that CREB-deficient mutant mice are sill able to display the normal behavioral responses to antidepressants. Accordingly, this indicates that there is a CREB-independent pathway for some of the pharmacologic actions of antidepressants. Activation of CREB and BDNF is hypothesized to promote cell survival by activating pro-survival and anti-apoptotic pathways, as well as having direct and indirect effects on cellular proliferation.

Insulin-like growth factor-1 (IGF-1) has recently become implicated in regulation of adult hippocampal neurogenesis [92]. IGF-1 and its corresponding receptors are found ubiquitously in the developing brain, but in the adult brain are found in cells in the hippocampus and olfactory bulb, both of which are areas where adult neurogenesis occurs [93]. Both central [94, 95] and systemic administration of IGF-1 [96] produces an increase in cell proliferation in the SGZ. Systemic IGF-1 produces a net increase in neurons not only by increasing cell proliferation but also by affecting cell fate or differentiation [96]. This effect on differentiation is another unique property of this growth factor.

In vitro, IGF-1 has a direct proliferative effect in adult hippocampal progenitor cells [97] *via* MAP kinase activation. IGF-1 has also been shown to prevent apoptosis in PC12 cells by activating the promoter of the anti-apoptotic factor bcl-2, and this proceeds in part through a novel signaling pathway involving MAPK kinase 6/p38b MAPK/ MAPKAP-K3 and requires CREB [98]. In this study, treatment of cells with IGF-1 also increased CREB phosphorylation. This suggests that there is an interaction between neurotrophic factors to increase cell survival.

In addition to trophic factors affecting proliferation and neurogenesis, they may also directly affect the local environment around the cells. Monje *et al*. [72] have provided evidence that in the SGZ, the local microenvironment regulates cellular differentiation. In this study, rats were given x-ray irradiation to produce ablation of the neural precursors. When grafts of healthy, non-irradiated stem cell cultures were implanted into the SGZ of irradiated animals, there was an inability of the transplanted cells to differentiate into neurons. This indicates that x-ray irradiation induced a dysfunction in the local microenvironment that prevented normal neurogenesis of the healthy grafted stem cell cultures. This regulation of neurogenesis by the local microenvironment may be another level of trophic regulation.

Taken together, these studies indicate that trophic factors may play an important role in hippocampal proliferation and neurogenesis. In conclusion, trophic factors may be working either independently or in concert to produce a supportive environment that maintains a normal level of cell birth, development and survival in the adult brain.

THE SEARCH FOR NEW ANTIDEPRESSANTS

Preclinical and clinical data indicate that reduced hippocampal neurogenesis may be seen in depressive disorders, and that antidepressants may produce their therapeutic effects by reversing this reduction in neurogenesis. There is ample evidence that many factors that are activated or affected by antidepressants are themselves neurogenic or play a role in neurogenic pathways. The current antidepressants that target monoamine neurotransmitter systems may activate CREB and BDNF and potentially other neurotrophic factors not yet known.

We hypothesize that the next wave of antidepressant drugs will, in addition to their effects on known transmitter or second messenger systems, involve either direct or indirect targeting of neurogenic factors. The future generation of antidepressant drugs may act both on proliferating cells as well on the cells' local microenvironment. This may result from activating not just one but multiple factors or pathways involved in the proliferation, survival and maintenance of proliferating cells and their trophic environment.

The further targeting of increased hippocampal cell proliferation as a preclinical endpoint may provide an antidepressant with greater efficacy, a better side effect profile, or a shorter onset to therapeutic efficacy then is currently available. In addition, the ability of novel compounds to be tested for neurogenic potential may become an additional way to evaluate a compound for putative antidepressant effects. Given the speed in which the field of hippocampal neurogenesis has advanced, it can be predicted that great strides in our understanding of both baseline and antidepressant-induced neurogenesis will occur in the next few years which will in turn produce a host of novel antidepressant drugs.

REFERENCES

References 99-101 are related articles recently published in Current Pharmaceutical Design.

[1] 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(23): 3095-105.

[2] Ballas C, Staab JP, Evans DL. Strategies for treatment-resistant depression. Psychopharmacol Bull 2002; 36(4 Suppl 3): 39-62.

[3] Malberg JE, Eisch AJ, Nestler EJ, Duman RS. Chronic antidepressant treatment increases neurogenesis in adult rat hippocampus. J Neurosci 2000; 20(24): 9104-10.

[4] Cajal Ry. Degeneration and regeneration of the nervous system. Haffner Publishing Co.: New York, NY. 1928; p. 750.

[5] Gould E, Gross CG. Neurogenesis in adult mammals: some progress and problems. J Neurosci 2002; 22(3): 619-23.

[6] Kaplan MS, Hinds JW. Neurogenesis in the adult rat: electron microscopic analysis of light radioautographs. Science 1977; 197(4308): 1092-4.

[7] Altman J, Das GD. Autoradiographic and histological evidence of postnatal hippocampal neurogenesis in rats. J Comp Neurol 1965; 124(3): 319-35.

[8] Takahashi T, Nowakowski RS, Caviness VS, Jr. BUdR as an S-phase marker for quantitative studies of cytokinetic behaviour in the murine cerebral ventricular zone. J Neurocytol 1992; 21(3): 185-97.

[9] Miller MW, Nowakowski RS. Use of bromodeoxyuridine-immunohistochemistry to examine the proliferation, migration and time of origin of cells in the central nervous system. Brain Res 1988; 457(1): 44-52.

[10] 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(2): 391-9.

[11] Cameron HA, McKay RD. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. J Comp Neurol 2001; 435(4): 406-17.

[12] Wennstrom M, Hellsten J, Ekdahl CT, Tingstrom A. Electroconvulsive seizures induce proliferation of NG2-expressing glial cells in adult rat hippocampus. Biol Psychiatry 2003; 54(10): 1015-24.

[13] Nakagawa S, Kim JE, Lee R, Chen J, Fujioka T, Malberg J, *et al*. Localization of phosphorylated cAMP response element-binding protein in immature neurons of adult hippocampus. J Neurosci 2002; 22(22): 9868-76.

[14] Palmer TD, Willhoite AR, Gage FH. Vascular niche for adult hippocampal neurogenesis. J Comp Neurol 2000; 425(4): 479-94.

[15] Malberg JE, Duman RS. Cell proliferation in adult hippocampus is decreased by inescapable stress: Reversal by fluoxetine treatment. Neuropsychopharmacology 2003; In press.

[16] Hastings NB, Gould E. Rapid extension of axons into the CA3 region by adult-generated granule cells. J Comp Neurol 1999; 413(1): 146-54.

[17] van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, Gage FH. Functional neurogenesis in the adult hippocampus. Nature 2002; 415(6875): 1030-4.

[18] West MJ, Slomianka L, Gundersen HJ. Unbiased stereological estimation of the total number of neurons in thesubdivisions of the rat hippocampus using the optical fractionator. Anat Rec 1991; 231(4): 482-97.

[19] Eisch AJ. Adult neurogenesis: implications for psychiatry. Prog Brain Res 2002; 138: 315-42.

[20] Nowakowski RS, Lewin SB, Miller MW. Bromodeoxyuridine immunohistochemical determination of the lengths of the cell cycle and the DNA-synthetic phase for an anatomically defined population. J Neurocytol 1989; 18(3): 311-8.

[21] Kaplan MS. Environment complexity stimulates visual cortex neurogenesis: death of a dogma and a research career. Trends Neurosci 2001; 24(10): 617-20.

[22] Calof AL, Rim PC, Askins KJ, Mumm JS, Gordon MK, Iannuzzelli P, *et al.* Factors regulating neurogenesis and programmed cell death in mouse olfactory epithelium. Ann N Y Acad Sci 1998; 855: 226-9.

[23] Goldman SA, Nottebohm F. Neuronal production, migration, and differentiation in a vocal control nucleus of the adult female canary brain. Proc Natl Acad Sci USA 1983; 80(8): 2390-4.

[24] Cameron HA, Woolley CS, McEwen BS, Gould E. Differentiation of newly born neurons and glia in the dentate gyrus of the adult rat. Neuroscience 1993; 56(2): 337-44.

[25] Cameron HA, Gould E. Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. Neuroscience 1994; 61(2): 203-9.

[26] Gould E, McEwen BS, Tanapat P, Galea LA, Fuchs E. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. J Neurosci 1997; 17(7): 2492-8.

[27] Gould E, Tanapat P, McEwen BS, Flugge G, Fuchs E. Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress. Proc Natl Acad Sci USA 1998; 95(6): 3168-71.

[28] Kempermann G, Kuhn HG, Gage FH. More hippocampal neurons in adult mice living in an enriched environment. Nature 1997; 386(6624): 493-5.

[29] Gould E, Reeves AJ, Fallah M, Tanapat P, Gross CG, Fuchs E. Hippocampal neurogenesis in adult Old World primates. Proc Natl Acad Sci USA 1999; 96(9): 5263-7.

[30] Eriksson PS, Perfilieva E, Bjork-Eriksson T, Alborn AM, Nordborg C, Peterson DA, *et al*. Neurogenesis in the adult human hippocampus. Nat Med 1998; 4(11): 1313-7.

[31] Gould E, Vail N, Wagers M, Gross CG. Adult-generated hippocampal and neocortical neurons in macaques have a transient existence. Proc Natl Acad Sci USA 2001; 98(19): 10910-7.

[32] Gould E, Reeves AJ, Graziano MS, Gross CG. Neurogenesis in the neocortex of adult primates. Science 1999; 286(5439): 548-52.

[33] Rakic P. Neurogenesis in adult primate neocortex: an evaluation of the evidence. Nat Rev Neurosci 2002; 3(1): 65-71.

[34] Kempermann G. Regulation of adult hippocampal neurogenesis - implications for novel theories of major depression. Bipolar Disord 2002; 4(1): 17-33.

[35] Duman RS, Nakagawa S, Malberg J. Regulation of adult neurogenesis by antidepressant treatment. Neuropsychopharmacology 2001; 25(6): 836-44.

[36] Tanapat P, Galea LA, Gould E. Stress inhibits the proliferation of granule cell precursors in the developing dentate gyrus. Int J Dev Neurosci 1998; 16(3-4): 235-9.

[37] Tanapat P, Hastings NB, Rydel TA, Galea LA, Gould E. Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. J Comp Neurol 2001; 437(4): 496-504.

[38] Pham K, Nacher J, Hof PR, McEwen BS. Repeated restraint stress suppresses neurogenesis and induces biphasic PSA-NCAM expression in the adult rat dentate gyrus. Eur J Neurosci 2003; 17(4): 879-86.

[39] Fuchs E, Flugge G. Stress, glucocorticoids and structural plasticity of the hippocampus. Neurosci Biobehav Rev 1998; 23(2): 295-300.

[40] Cameron HA, Woolley CS, Gould E. Adrenal steroid receptor immunoreactivity in cells born in the adult rat dentate gyrus. Brain Res 1993; 611(2): 342-6.

[41] Shors TJ, Miesegaes G, Beylin A, Zhao M, Rydel T, Gould E. Neurogenesis in the adult is involved in the formation of trace memories. Nature 2001; 410(6826): 372-6.

[42] Kim JJ, Diamond DM. The stressed hippocampus, synaptic plasticity and lost memories. Nat Rev Neurosci 2002; 3(6): 453-62.

[43] Czeh B, Welt T, Fischer AK, Erhardt A, Schmitt W, Muller MB, *et al*. Chronic psychosocial stress and concomitant repetitive transcranial magnetic stimulation: effects on stress hormone levels and adult hippocampal neurogenesis. Biol Psychiatry 2002; 52(11): 1057-65.

[44] Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW. Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci USA 1996; 93(9): 3908-13.

[45] Magarinos AM, Deslandes A, McEwen BS. Effects of antidepressants and benzodiazepine treatments on the dendritic structure of CA3 pyramidal neurons after chronic stress. Eur J Pharmacol 1999; 371(2-3): 113-22.

[46] McEwen BS. Stress and hippocampal plasticity. Annu Rev Neurosci 1999; 22: 105-22.

[47] Watanabe Y, Gould E, Daniels DC, Cameron H, McEwen BS. Tianeptine attenuates stress-induced morphological changes in the hippocampus. Eur J Pharmacol 1992; 222: 157-162.

[48] Bierhaus A, Wolf J, Andrassy M, Rohleder N, Humpert PM, Petrov D, *et al*. A mechanism converting psychosocial stress into mononuclear cell activation. Proc Natl Acad Sci USA 2003; 100(4): 1920-5.

[49] Madsen TM, Treschow A, Bengzon J, Bolwig TG, Lindvall O, Tingstrom A. Increased neurogenesis in a model of electroconvulsive therapy. Biol Psychiatry 2000; 47(12): 1043-9.

[50] 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(5634): 805-9.

[51] 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. J Neurosci 2002; 22(9): 3673-82.

[52] Chen G, Rajkowska G, Du F, Seraji-Bozorgzad N, Manji HK. Enhancement of hippocampal neurogenesis by lithium. J Neurochem 2000; 75(4): 1729-34.

[53] Ende G, Braus DF, Walter S, Weber-Fahr W, Henn FA. The hippocampus in patients treated with electroconvulsive therapy: a proton magnetic resonance spectroscopic imaging study. Arch Gen Psychiatry 2000; 57(10): 937-43.

[54] Eisch AJ, Barrot M, Schad CA, Self DW, Nestler EJ. Opiates inhibit neurogenesis in the adult rat hippocampus. Proc Natl Acad Sci USA 2000; 97(13): 7579-84.

[55] Bai F, Bergeron M, Nelson DL. Chronic AMPA receptor potentiator (LY451646) treatment increases cell proliferation in adult rat hippocampus. Neuropharmacology 2003; 44(8): 1013-21.

[56] Rajkowska G, Miguel-Hidalgo JJ, Wei J, Dilley G, Pittman SD, Meltzer HY, *et al*. Morphometric evidence for neuronal and glial prefrontal cell pathology in major depression. Biol Psychiatry 1999; 45(9): 1085-98.

[57] Sheline YI. Neuroimaging studies of mood disorder effects on the brain. Biol Psychiatry 2003; 54(3): 338-52.

[58] Drevets WC. Functional anatomical abnormalities in limbic and prefrontal cortical structures in major depression. Prog Brain Res 2000; 126: 413-31.

[59] Sheline YI, Sanghavi M, Mintun MA, Gado MH. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. J Neurosci 1999; 19(12): 5034-43.

[60] Sheline YI, Gado MH, Kraemer HC. Untreated depression and hippocampal volume loss. Am J Psychiatry 2003; 160(8): 1516-8.

[61] Vermetten E, Vythilingam M, Southwick SM, Charney DS, Bremner JD. Long-term treatment with paroxetine increases verbal declarative memory and hippocampal volume in posttraumatic stress disorder. Biol Psychiatry 2003; 54(7): 693-702.

[62] Cryan JF, Markou A, Lucki I. Assessing antidepressant activity in rodents: recent developments and future needs. Trends Pharmacol Sci 2002; 23(5): 238-45.

[63] Fuchs E, Flugge G, Ohl F, Lucassen P, Vollmann-Honsdorf GK, Michaelis T. Psychosocial stress, glucocorticoids, and structural alterations in the tree shrew hippocampus. Physiol Behav 2001; 73(3): 285-91.

[64] Thiebot MH, Martin P, Puech AJ. Animal behavioural studies in the evaluation of antidepressant drugs. Br J Psychiatry Suppl 1992(15): 44-50.

[65] van der Hart MG, Czeh B, de Biurrun G, Michaelis T, Watanabe T, Natt O, *et al*. Substance P receptor antagonist and clomipramine prevent stress-induced alterations in cerebral metabolites, cytogenesis in the dentate gyrus and hippocampal volume. Mol Psychiatry 2002; 7(9): 933-41.

[66] 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 USA 2001; 98(22): 12796-801.

[67] Porter RJ, Gallagher P, Thompson JM, Young AH. Neurocognitive impairment in drug-free patients with major depressive disorder. Br J Psychiatry 2003; 182: 214-20.

[68] Madsen TM, Kristjansen PE, Bolwig TG, Wortwein G. Arrested neuronal proliferation and impaired hippocampal function following fractionated brain irradiation in the adult rat. Neuroscience 2003; 119(3): 635-42.

[69] 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(5): 578-84.

[70] Tada E, Parent JM, Lowenstein DH, Fike JR. X-irradiation causes a prolonged reduction in cell proliferation in the dentate gyrus of adult rats. Neuroscience 2000; 99(1): 33-41.

[71] Mizumatsu S, Monje ML, Morhardt DR, Rola R, Palmer TD, Fike JR. Extreme sensitivity of adult neurogenesis to low doses of X-irradiation. Cancer Res 2003; 63(14): 4021-7.

[72] Monje ML, Mizumatsu S, Fike JR, Palmer TD. Irradiation induces neural precursor-cell dysfunction. Nat Med 2002; 8(9): 955-62.

[73] Dimberg Y, Vazquez M, Soderstrom S, Ebendal T. Effects of X-irradiation on nerve growth factor in the developing mouse brain. Toxicol Lett 1997; 90(1): 35-43.

[74] Brezun JM, Daszuta A. Depletion in serotonin decreases neurogenesis in the dentate gyrus and the subventricular zone of adult rats. Neuroscience 1999; 89(4): 999-1002.

[75] Brezun JM, Daszuta A. Serotonergic reinnervation reverses lesion-induced decreases in PSA-NCAM labeling and proliferation of hippocampal cells in adult rats. Hippocampus 2000; 10(1): 37-46.

[76] Brezun JM, Daszuta A. Serotonin may stimulate granule cell proliferation in the adult hippocampus, as observed in rats grafted with foetal raphe neurons. Eur J Neurosci 2000; 12(1): 391-6.

[77] Jacobs BL. Adult brain neurogenesis and depression. Brain Behav Immun 2002; 16(5): 602-9.

[78] Radley JJ, Jacobs BL. 5-HT1A receptor antagonist administration decreases cell proliferation in the dentate gyrus. Brain Res 2002; 955(1-2): 264-7.

[79] Veenstra-VanderWeele J, Anderson GM, Cook EH Jr. Pharmacogenetics and the serotonin system: initial studies and future directions. Eur J Pharmacol 2000; 410(2-3): 165-181.

[80] Kulkarni VA, Jha S, Vaidya VA. Depletion of norepinephrine decreases the proliferation, but does not influence the survival and differentiation, of granule cell progenitors in the adult rat hippocampus. Eur J Neurosci 2002; 16(10): 2008-12.

[81] Laifenfeld D, Klein E, Ben-Shachar D. Norepinephrine alters the expression of genes involved in neuronal sprouting and differentiation: relevance for major depression and antidepressant mechanisms. J Neurochem 2002; 83(5): 1054-64.

[82] Nibuya M, Morinobu S, Duman RS. Regulation of BDNF and trkB mRNA in rat brain by chronic electroconvulsive seizure and antidepressant drug treatments. J Neurosci 1995; 15(11): 7539-47.

[83] Chen B, Dowlatshahi D, MacQueen GM, Wang JF, Young LT. Increased hippocampal BDNF immunoreactivity in subjects treated with antidepressant medication. Biol Psychiatry 2001; 50(4): 260-5.

[84] Siuciak JA, Lewis DR, Wiegand SJ, Lindsay RM. Antidepressant-like effect of brain-derived neurotrophic factor (BDNF). Pharmacol Biochem Behav 1997; 56(1): 131-7.

[85] Shirayama Y, Chen AC, Nakagawa S, Russell DS, Duman RS. Brain-derived neurotrophic factor produces antidepressant effects in behavioral models of depression. J Neurosci 2002; 22(8): 3251-61.

[86] Zigova T, Pencea V, Wiegand SJ, Luskin MB. Intraventricular administration of BDNF increases the number of newly generated neurons in the adult olfactory bulb. Mol Cell Neurosci 1998; 11(4): 234-45.

[87] Pencea V, Bingaman KD, Wiegand SJ, Luskin MB. Infusion of brain-derived neurotrophic factor into the lateral ventricle of the adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus. J Neurosci 2001; 21(17): 6706-17.

[88] D'Sa C, Duman RS. Antidepressants and neuroplasticity. Bipolar Disord 2002; 4(3): 183-94.

[89] Duman RS, Malberg J, Thome J. Neural plasticity to stress and antidepressant treatment. Biol Psychiatry 1999; 46(9): 1181-91.

[90] Takahashi M, Terwilliger R, Lane C, Mezes PS, Conti M, Duman RS. Chronic antidepressant administration increases the expression of cAMP-specific phosphodiesterase 4A and 4B isoforms. J Neurosci 1999; 19(2): 610-8.

[91] Conti AC, Cryan JF, Dalvi A, Lucki I, Blendy JA. cAMP response element-binding protein is essential for the upregulation of brain-derived neurotrophic factor transcription, but not the behavioral or endocrine responses to antidepressant drugs. J Neurosci 2002; 22(8): 3262-8.

[92] Anderson MF, Aberg MA, Nilsson M, Eriksson PS. Insulin-like growth factor-I and neurogenesis in the adult mammalian brain. Brain Res Dev Brain Res 2002; 134(1-2): 115-22.

[93] Anlar B, Sullivan KA, Feldman EL. Insulin-like growth factor-I and central nervous system development. Horm Metab Res 1999; 31: 120-125.

[94] Lichtenwalner RJ, Forbes ME, Bennett SA, Lynch CD, Sonntag WE, Riddle DR. Intracerebroventricular infusion of insulin-like growth factor-I ameliorates the age-related decline in hippocampal neurogenesis. Neuroscience 2001; 107(4): 603-13.

[95] Poe BH, Linville C, Riddle DR, Sonntag WE, Brunso-Bechtold JK. Effects of age and insulin-like growth factor-1 on neuron and synapse numbers in area CA3 of hippocampus. Neuroscience 2001; 107(2): 231-8.

[96] Aberg MA, Aberg ND, Hedbacker H, Oscarsson J, Eriksson PS. Peripheral infusion of IGF-I selectively induces neurogenesis in the adult rat hippocampus. J Neurosci 2000; 20(8): 2896-903.

[97] Aberg MA, Aberg ND, Palmer TD, Alborn AM, Carlsson-Skwirut C, Bang P, *et al*. IGF-I has a direct proliferative effect in adult hippocampal progenitor cells. Mol Cell Neurosci 2003; 24(1): 23-40.

[98] Pugazhenthi S, Miller E, Sable C, Young P, Heidenreich KA, Boxer LM, *et al*. Insulin-like growth factor-I induces bcl-2 promoter through the transcription factor cAMP-response element-binding protein. J Biol Chem 1999; 274(39): 27529-35.

[99] Blum J, Handmaker H, Rinne NA. Technetium labeled small peptide radiopharmaceuticals in the identification of lung cancer. Curr Pharm Design 2002; 8(20): 1827-36.

[100] Hartvig P, Bergstrom M, Antoni G, Langstrom B. Positron emission tomography and brain monoamine neurotransmission -- entries for study of drug interactions. Curr Pharm Design 2002; 8(16): 1417-34.

[101] Schechter LE, Dawson LA, Harder JA. The potential utility of 5-HT1A receptor antagonists in the treatment of cognitive dysfunction associated with Alzheimer s disease. Curr Pharm Design 2002; 8(2): 139-45.