Implications of adult hippocampal neurogenesis in antidepressant action

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In the dentate gyrus of the hippocampus, cell birth and maturation into neurons, or neurogenesis, occur throughout the lifetime of animals and humans. Multiple factors have been shown to regulate adult neurogenesis, and a number of findings in this field have had a large impact on basic and clinical research in depression. It has been reported that both physical and psychosocial stress paradigms, as well as some animal models of depression, produce a decrease in hippocampal cell proliferation and neurogenesis. Conversely, long-term, but not short-term, treatment with different classes of antidepressant drug increases cell proliferation and neurogenesis. Patients with depressive disorders or post-traumatic stress disorder have reduced hippocampal volume. Given this interaction of stress, depression and neurogenesis, a current hypothesis is that reduced adult hippocampal cell proliferation or neurogenesis may be involved in the pathophysiology of depression and that reversal or prevention of the decrease in neurogenesis may be one way in which the antidepressant drugs exert their effects. Research from this emerging field will further our understanding of the effects of stress and depression on the brain and the mechanism of action of antidepressant drugs.

Dans le gyrus denté de l'hippocampe, la naissance des cellules et leur maturation en neurones, ou neurogenèse, se produisent pendant toute la vie des animaux et des êtres humains. On a démontré que de multiples facteurs régularisent la neurogenèse chez l'adulte, et de nombreuses constatations dans ce domaine ont eu un effet important sur la recherche fondamentale et clinique sur la dépression. On a signalé que des paradigmes de stress à la fois physique et psychosocial, ainsi que certains modèles animaux de dépression, entraînent une diminution de la prolifération des cellules dans l'hippocampe et une baisse de la neurogenèse. Par ailleurs, le traitement avec différentes catégories d'antidépresseurs augmente la prolifération cellulaire et la neurogenèse pendant la durée d'un traitement chronique, mais non pendant la durée d'un traitement de plus courte durée. Le volume de l'hippocampe diminue chez les patients atteints de troubles dépressifs ou du syndrome de stress posttraumatique. Compte tenu de cette interaction entre le stress, la dépression et la neurogenèse, on pose actuellement comme hypothèse que la réduction de la prolifération des cellules ou de la neurogenèse dans l'hippocampe de l'adulte peut jouer un rôle dans la pathophysiologie de la dépression et que l'inversion et la prévention de la diminution de la neurogenèse peuvent être un moyen d'action des antidépresseurs. La recherche qui émanera de ce nouveau domaine améliorera notre compréhension des effets du stress et de la dépression sur le cerveau et le mode d'action des antidépresseurs.

Introduction

The relatively recent finding that the birth of new neurons (neurogenesis) occurs in the hippocampal formation throughout the lifespan of mammals and humans has changed the way we think about the adult brain

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and central nervous system (CNS) disease. Although cell proliferation and neurogenesis had been previously identified in the olfactory bulb and the subventricular zone of the lateral ventricle,[1] the idea that cells were continually born in the adult hippocampus was not accepted until relatively recently.[2] The search for factors that regulate adult hippocampal neurogenesis has produced a large and varied field of study, and a number of findings have had a significant impact on basic and clinical research in depression.

This review begins with a short introduction to the field of neurogenesis and then presents preclinical and clinical evidence that stress and depression downregulate and antidepressant drugs upregulate hippocampal neurogenesis. Importantly, the first direct evidence has recently been published by Santarelli et al[3] that de novo cell proliferation is necessary for antidepressant action.

The hypothesis will be explored that depression may result from exposure to chronic stress, which in turn leads to cellular dysfunction,[4,5] one aspect of which may be decreased adult hippocampal neurogenesis. These stress-induced changes lead to a net loss of synaptic plasticity, which may manifest itself in depressive symptomatology. Among the mechanisms by which antidepressant drugs may exert their effect is by increasing cell proliferation in order to reverse or offset the deleterious effects of stress on the brain.

Defining neurogenesis and cell proliferation

Cell proliferation and neurogenesis have been identified in the dentate gyrus of adult animals in a number of different species, including the rat, mouse, guinea pig, primates and humans.[6–10] The proliferating cells, born in the subgranular zone and hilus of the dentate gyrus, mature into neurons and make functional synaptic connections with the hippocampal circuitry.[11,12] The earliest studies used [^{3}H]-thymidine to label the dividing cells,[13,14] whereas the nonradioactive thymidine analogue bromodeoxyuridine (BrdU) is now most commonly used. BrdU can be injected intraperitoneally where it crosses the blood–brain barrier and is taken up into the DNA of cells during the S phase of mitosis. After BrdU injection, the animals are sacrificed at different time points and brain slices are stained for BrdU using immunohistochemistry. A BrdU-positive cell is determined to be an indicator of a newly born cell and the numbers of BrdU-positive cells are quantified, often using a modified stereology protocol.[15,16] As the cells mature, they differentiate into neurons or glia. For determination of the phenotype of the BrdU-positive cells, double or triple immunohistochemical labelling is used. At short intervals after BrdU injection, the BrdU-positive cells express immature neuronal and glial markers, whereas at later time points antibodies for cell migration and mature cells are used to determine the final fate of the BrdU-positive cells.[17]

There are many issues surrounding the use of BrdU and quantification of the cells.[18,19] One of the critical issues when comparing papers within the field is the use of the word "proliferation" versus "neurogenesis." In strict terms, cell proliferation is defined as 1 round of cell division that can be measured after BrdU injection. In contrast, neurogenesis relates to the maturation of BrdU-positive cells and differentiation into mature neuronal or glial phenotypes. A third type of study investigates the effect of experimental manipulations, which are administered after the BrdU has been injected, on the survival and differentiation of cells. Although these studies are referred to on the whole as "neurogenesis studies," the experimental design is very different for determination of cell proliferation, neurogenesis and survival.

In order to study the effect of a drug or behavioural treatment on cell proliferation, it is necessary to define the time at which the animals are sacrificed after the BrdU injection in order for the BrdU cell counts to represent 1 round of cell division. Cameron and McKay[20] have elegantly demonstrated that the S phase of cell division in a healthy adult rat is about 2 hours. Therefore, the BrdU counts obtained after sacrificing an animal 2 hours after a single BrdU injection represent 1 round of cell proliferation. However, this distinction is not often made, and in many published studies the word proliferation is used after multiple injections of BrdU and different time points of sacrifice after injection. In addition, an issue arises in that the drug treatment before the BrdU injection may affect the length of the animals' cell cycle and S phase, or affect the amount of cell death that occurs after the BrdU injection.[21] Any alteration of variables in the cell cycle or rate of apoptosis between the treatment and control groups can produce confounding and subsequent misinterpretation of the results.

It should be kept in mind that variables that affect cell proliferation do not always affect neurogenesis and survival of cells, and care must be taken in the interpretation of experiments where only 1 manipulation is performed. It is also necessary to understand that the time course of BrdU injection and subsequent sacrifice

can have a large effect on the interpretation of and conclusions from the presented data. This is especially important when comparing experiments and when trying to replicate or extend the results of published work. It should also be stressed that although hippocampal neurogenesis has been demonstrated in almost all species investigated, including primates (albeit at a much reduced level than is seen in lower primates and rodents), additional reports of cell proliferation and neurogenesis in cortical areas are still being investigated and debated by many prominent researchers.[22–24] Although the effects of depression and antidepressant effects are by no means limited to the hippocampus, this review will focus on hippocampal and not cortical cell proliferation and neurogenesis.

Stress and neurogenesis

A large number of variables regulate adult hippocampal cell proliferation and neurogenesis such as age, strain, gender, hormones, environment, exercise and learning.[25] One of the most consistent reports throughout the literature is that acute and chronic stress produce a decrease in cell proliferation and neurogenesis.

These findings are of considerable interest to both basic and clinical researchers studying depression. This is because there are a large number of stress-based hypotheses of depression,[26] and almost all of the animal models of depression have a stress component.[27]

Multiple acute and chronic physical and psychosocial stress paradigms have been shown to reduce cell proliferation and neurogenesis. In the rat, a single exposure to predator odour decreases cell proliferation, as demonstrated by using either [^{3}H]-thymidine or BrdU as a marker for cell proliferation.[28,29] Acute exposure to inescapable shock produces a decrease in BrdU cell counts that is still detectable when the BrdU is injected 7 days after the exposure to shock.[30] Pham et al[31] have demonstrated that restraint stress negatively affects cell proliferation, but a longer period of exposure (21 days) is necessary to produce downregulation.

A single 1-hour exposure to a psychosocial stressor, the resident–intruder paradigm, reduces cell proliferation and neurogenesis in marmosets and tree shrews.[32,33] Chronic psychosocial stress paradigms also produce decreases in cell proliferation. Exposure to 35 days of the social defeat paradigm caused a decrease in hippocampal cell proliferation in tree shrews, along with decreases in hippocampal volume and cerebral metabolite levels.[34] Similarly, rats exposed to 18 days of a social defeat paradigm showed a decrease in cell proliferation.[35]

Although 1 study has reported that acute stressors affect both cell proliferation and neurogenesis,[32] other studies have demonstrated that a stress-induced downregulation of cell proliferation does not always translate into a decrease in neurogenesis. For example, rats exposed to inescapable shock had a decrease in BrdU-positive cells when sacrificed 2 hours after a BrdU injection (proliferation) compared with nonshocked controls, but no change in staining was detected when animals were sacrificed 28 days after BrdU administration.[30] This is in agreement with the findings of Tanapat et al,[29] who reported a stress-induced decrease in cell proliferation at short time points after BrdU administration (1 and 7 days) but no effect of stress on the number of cells at a later time point (28 days). This indicates that a compensatory change may occur between the 2 time points investigated.[30] In this case, in the animals exposed to inescapable shock stress, the compensatory change may have induced a greater number of BrdU-positive cells to survive to the later time point, compared with the percentage that survived in control animals. Therefore, in these animals, fewer cells were born compared with nonstressed controls, but a greater percentage survived to the 28-day time point. This compensatory effect produces the same net number of cells at a later time point.

Stress has also been shown to affect cell survival. It has been shown that 18 days' exposure to the social defeat paradigm after BrdU injection decreased cell survival in the rat.[35] In addition, 3 or 6 weeks of restraint stress after BrdU administration produced a decrease in BrdU-positive cell numbers.[31] In contrast, a single week of restraint at either days 4–9 or days 10–17 after BrdU injection was not sufficient to decrease the number of BrdU-positive cells.[36] Taken together, these studies indicate that, in contrast to the short-term effects of stress on cell proliferation, long-term exposure to a stressor is necessary to affect cell survival.

On the basis of these studies, it can be concluded that both acute and chronic stress have effects on cell proliferation, survival and neurogenesis. To date, there is no reported effect of stress on differentiation of BrdU-positive cells into mature phenotypes. Chronic exposure to stress, which may be more applicable to a real-world situation than an acute stressor paradigm, may work through multiple mechanisms to regulate neurogenesis. One hypothesis is that the cumulative effects of stress on

proliferation, neurogenesis and survival produce the changes in dendritic remodelling that may be involved in the pathophysiology of chronic depressive disorder.

Other stressors, such as prenatal stress, have also been shown to produce a decrease in cell proliferation in the adult rat.[37,38] This has been shown to be a gender-specific effect, with female rats being more sensitive to some of the effects of stress on cell proliferation and dendritic spine density.[39,40] The incidence of depression is higher in females than males,[41] and estrogen has been shown to be a regulator of cell proliferation.[42] Clearly, there may be important functional differences in the stress and cell proliferation responses between males and females. However, most basic research studies have been performed on male rats. Further studies investigating sex-specific differences are needed, and caution must be used when extrapolating data from males to females.

The deleterious effect of stress on the brain is not specific to hippocampal cell proliferation. Before studies of neurogenesis were performed, it was well established that stress affected brain restructuring and reorganization in the adult animal.[43,44] Repeated stress results in atrophy of CA3 pyramidal neurons,[45,46] which is reversed by the atypical antidepressant tianeptine.[47] Chronic social stress also reduces dendritic arborization of CA3 neurons.[48]

It has been proposed by McEwen[43] and by Sapolsky[49] that the effect of stress on the dentate gyrus granule cells, as well as CA3 pyramidal neurons, may be the result of hypersecretion of glucocorticoids (GCs) such as corticosterone (CORT) and overproduction of excitatory amino acids. Stress increases serum bound and free CORT levels,[46] and exogenous administration of CORT produces a decrease in cell proliferation that can be prevented by the *N*-methyl-D-aspartate (NMDA) antagonist MK-801.[50] CORT-induced decreases in cell proliferation can also be prevented by administering the dehydroepiandrosterone steroid (DHEA) or by performing adrenalectomy on the animals.[29]

All of the stress paradigms mentioned here also increase CORT levels in intact animals. This stress-induced increase in CORT may be responsible for the decrease in cell proliferation. Although CORT is a potent downregulator of cell proliferation, a study using [^{3}H]-thymidine autoradiography combined with immunohistochemistry has shown that newborn hippocampal cells have neither type I nor type II GC receptors.[51] Therefore, although CORT is released during stress and cell proliferation is decreased, this is not because of a direct effect of CORT on the cells but must occur as a result of an additional regulatory pathway on the hippocampal stem cells. In addition, one study has demonstrated that decreases in cell proliferation can be seen even in the absence of elevated CORT levels.[30] The many factors underlying stress-induced effects on hippocampal reorganization and hippocampal cell proliferation are currently being studied.

Although stress decreases cell proliferation, this downregulation is not solely responsible for depressive symptoms and the pathophysiology of depression. Stress and depression constitute a multifaceted picture, and decreased cell proliferation is merely 1 part of a long path leading from the first stress exposure to the manifestation of depression.[52] The response of the individual organism to a stressful (or thus perceived non-stressful) situation, along with a possible genetic predisposition to affective disorders, may also play a large role in stress-induced changes in cell proliferation and neurogenesis. The study of the effects of stress on neurogenesis is still an emerging field, and currently the mechanism by which decreased cell proliferation is associated with depressive symptoms is unknown.

Antidepressants and neurogenesis

In contrast to stress-induced downregulation of hippocampal neurogenesis, antidepressant treatment has been shown to increase cell proliferation and neurogenesis. Fluoxetine (specific serotonin reuptake inhibitor), tranylcypromine (monoamine oxidase inhibitor), reboxetine (specific norepinephrine reuptake inhibitor) and rolipram (phosphodiesterase-IV inhibitor) have all been shown to produce this effect. Short-term fluoxetine administration (1 or 5 d) had no effect on cell proliferation, whereas the fluoxetine-induced increase in cell proliferation was seen after 14 days of drug treatment.[16] These data indicate that a long-term antidepressant regimen is necessary to increase cell proliferation. There was no difference in cell proliferation between groups of animals treated with fluoxetine for 14 days or 28 days, indicating that there may be a plateau effect after 14 days.[16] Similarly, a single dose of rolipram did not affect cell proliferation; long-term treatment (21 d) was necessary to produce an increase in cell proliferation and neurogenesis.[53] This delayed onset of action corresponds to that necessary for the therapeutic action of antidepressants.[16]

Electroconvulsive shock therapy (ECT) also increases cell proliferation and neurogenesis in the adult rat.[16,54,55]

When compared with the chemical antidepressants, ECT is the most potent inducer of cell proliferation.[16] Although 1 study has reported that a single ECT exposure significantly increased cell proliferation, it has been shown that a longer duration of ECT treatment produces subsequent increases in cell proliferation.[54]

Almost all antidepressants investigated have been shown to increase cell proliferation and neurogenesis in a chronic time course. In addition to the compounds mentioned earlier, tianeptine, an atypical antidepressant, increases hippocampal cell proliferation and neurogenesis.[34] Although the effects have mainly been studied in vivo, fluoxetine has been shown to increase cell proliferation in vitro using cell cultures treated with a dose relevant to a therapeutic plasma concentration.[56] Only 1 antidepressant therapy, transcranial magnetic stimulation (TMS), has not been shown to affect cell proliferation. Although TMS has neuroprotective effects both in vitro and in vivo, the mechanism by which it acts is not clear.[35]

It has been shown that most BrdU-positive cells induced by antidepressant or ECT administration become neurons, as determined by double or triple labelling with mature neuronal markers.[16,53,54] A small percentage become glial-positive cells. No antidepressant treatments have been shown to alter the percentage of cells that differentiate into neurons or glia. It is hypothesized that the antidepressant drugs produce an increase in neurogenesis by increasing cell proliferation and that most of those newly born cells become neurons. Therefore, the net effect of the drugs is to increase neurogenesis.

The effect of antidepressants on cell survival has also been investigated. After BrdU injection, animals were given fluoxetine for either 14 or 28 days. After 14 days of fluoxetine, there was no increase in BrdU-positive cells, indicating that the same time point and dose that produced an increase in cell proliferation did not affect survival.[16] However, when fluoxetine was administered for 28 days after the BrdU injection, there was a 25% increase in the number of BrdU-positive cells, indicating that the additional 14 days of fluoxetine had an effect on cell survival.[17] This indicates that a longer time of antidepressant administration may be necessary to increase cell survival. We have also demonstrated that 3 weeks of rolipram given post-BrdU injection increased cell survival.[17]

The neurogenic effects of antidepressants have been shown to be specific to the hippocampus; antidepressant treatment did not affect BrdU staining in the subventricular zone of the lateral ventricle, which is another neurogenic region in the adult brain.[16,57] The increase in cell proliferation and neurogenesis was also specific to the antidepressant drugs. Long-term administration of haloperidol, a nonantidepressant psychotropic drug, did not produce a decrease in cell proliferation,[16] and this result has been replicated by other laboratories.[19]

Taken together, these studies have produced a large pool of data indicating that antidepressants increase neurogenesis. This is produced by the antidepressants acting on both cell proliferation and survival. Antidepressant treatment increases the number of cells born in the hippocampus (proliferation), and the majority of those cells differentiate into neurons. This creates a net effect of increased neurogenesis. After an increased number of new cells are born, antidepressants allow a greater percentage of those cells to survive and become neurons. In this way, a net effect of increased neurogenesis is again seen.

A number of investigators in drug development have hypothesized that demonstrating increased hippocampal neurogenesis by a novel compound is one way to validate that the novel compound may be an antidepressant.[58,59] Although antidepressant drugs constitute an immense market, there are still unmet medical needs such as improved efficacy (20%–30% of patients are resistant to current drug therapies)[60] and decreasing the current 2–3-week time lag from onset of treatment to therapeutic efficacy. Currently, the long time course needed to increase cell proliferation and neurogenesis parallels that seen in the therapeutic time lag. One hypothesis is that a reduction in time needed to increase proliferation and neurogenesis would result in a shorter time to clinical efficacy, although that hypothesis still needs to be experimentally determined. Another issue with analyzing novel compounds for their neurogenic qualities is that the time-intensive and labour-intensive cell counting procedure reduces the ability of 1 investigator or 1 group to analyze novel compounds quickly.

Antidepressant treatment reverses the stress-induced downregulation of neurogenesis

Many of the studies cited here have investigated the effect of antidepressant-induced neurogenesis on nonstressed animals. It may be argued that animals under-

going stress are better models in which to study the effects of antidepressants on neurogenesis, to determine whether the drugs prevent or reverse the deleterious effects of stress. To date, 2 animal models of depression, psychosocial stress and learned helplessness, have been used.

In tree shrews, chronic exposure to psychosocial conflict produced a decrease in cell proliferation, which is prevented by treatment with the atypical antidepressant tianeptine. Along with the reversal of the stress-induced decrease in cell proliferation, tianeptine also reversed the stress-induced decreases in hippocampal volume and decreases in cerebral metabolites.[34]

In the learned helplessness model of depression, exposure to inescapable shock produces subsequent behavioural deficits that can be reversed by antidepressant treatment.[27] In a recent study,[30] inescapable shock produced a decrease in cell proliferation when BrdU was injected 7 days after the shock. This decrease in cell proliferation was accompanied by behavioural deficits at this time point. Fluoxetine treatment prevented the downregulation in cell proliferation and also reversed the behavioural deficits. This indicates that in a commonly used model of depression, exposure to inescapable shock produces a long-term decrease of cell proliferation, which can be reversed by antidepressant treatment.

In addition to the animal models of depression, ECT has been shown to reverse the effects of stress on hippocampal cell proliferation. Multiple days of ECT have been shown to completely reverse a corticosterone-induced decrease in cell proliferation.[55] Antidepressants have been shown to prevent the negative effects of stress on other hippocampal functions as well. Stress-induced atrophy of CA3 pyramidal neurons in the hippocampus is reversed by tianeptine,[47] and antidepressant treatment also reverses the stress-induced downregulation of brain-derived neurotrophic factor (BDNF).[61]

It is clear from the studies cited here that there is an interaction of stress, antidepressant action and neurogenesis. Taken together, we hypothesize that the antidepressant-induced increase in the number and function of hippocampal granule cells could represent an important adaptive response to stress. This effect would be in opposition to the downregulation of neurogenesis, as well as atrophy of hippocampal neurons, which may occur in the pathophysiology of depression.

Clinical studies: from animal to human

Clinical evidence is emerging that hippocampal volume is decreased in affective disorders and that antidepressants reverse these decreases. It has been shown that hippocampal size is decreased in patients with major depressive disorder, post-traumatic stress disorder (PTSD) and Cushing's disease.[62–64] Multiple studies have been performed to document pathologic changes associated with depressive disorders.[65] Stereological studies in post-mortem tissue from depressed populations reveal decreased neuronal size, decreased neuronal and glial number, decreased cortical thickness, and decreased cerebral blood flow and glucose metabolism.[66–69]

More recently, imaging studies have reported that reduction in hippocampal volume is correlated with the length of depressive illness, indicating that the change in hippocampal volume may be caused by depression.[70,71] In support of this, it is reported that patients with multiple depressive episodes have decreases in hippocampal volume compared with patients experiencing their first depressive episode and compared with healthy controls.[72] Further studies have demonstrated that antidepressant treatment can reverse the depression-induced decreases in hippocampal volume.[73] Sheline et al[70] demonstrate that although decreased hippocampal volume is seen in untreated depressed patients, subjects treated with antidepressants have hippocampal volumes comparable to those of nondepressed controls. This indicates that antidepressants can reverse the depression-induced decreases in cell proliferation and normalize hippocampal size. Although relatively few new hippocampal cells are born daily in the adult human, the fact that imaging studies are able to detect decreases in depressed populations demonstrate that lifetime depression does have an effect on dendritic remodelling, leading to a decrease in the number of proliferating cells and granule cells with a net effect of decreased hippocampal size.

Recent imaging data demonstrate that CNS structural abnormalities are a consistent finding in patients with depressive disorders.[65] However, the evidence does not indicate whether the changes appeared before the onset of the disease or whether they were a direct cause of the disease. One recent study by Gilbertson et al[74] addressed this issue by looking at hippocampal volume in patients with PTSD and their identical twins. The patients with PTSD had smaller hippocam-

pal volume, but the unaffected identical twin also had reduced hippocampal volume, suggesting that the reduced volume may produce a state of vulnerability for this stress-related disorder.

The relation between hippocampal volume and illness is still being investigated. It is not known whether the reduced volume results from a decrease in the number of granule cells or other cell types. However, it may be that the decrease in volume comes not only from decreased cell proliferation but also from atrophy or increased apoptosis induced by stress.[75] Additional imaging and postmortem studies will be needed to address these questions, but the reduced hippocampal volume is consistent with the possibility that decreased neurogenesis or neuronal atrophy, or both, may occur in depressed patients. The hippocampal atrophy observed in patients with Cushing's disease is reversed by treatment, suggesting that this effect is reversible.[76] One hypothesis is that the antidepressant-induced upregulation of neurogenesis would act in a similar manner by reversing or opposing the reduction of hippocampal volume.

Neurotrophic factors

One way in which antidepressants may produce their behavioural and neurogenic effects is by increasing neurotrophic factors such as BDNF or insulin-like growth factor (IGF-1). Intracerebroventricular administration of BDNF increases neurogenesis,[77] and both intracerebroventricular and systemic administration of IGF-1 increase cell proliferation and neurogenesis.[78,79] IGF-1 also increases the differentiation of cells to neurons and is one of the few experimental manipulations that affect differentiation.

Long-term antidepressant treatment has been shown to increase BDNF protein and mRNA levels, and this treatment also reverses the stress-induced downregulation of BDNF.[61] However, it has also been reported that the effect of antidepressants on BNDF gene expression may be biphasic and time dependent.[80] Exogenous administration of BDNF, either by the intracerebroventricular or the intrahippocampal route, has antidepressant effects on multiple models of depression.[81,82] Clinically, there is increased BDNF immunoreactivity in subjects treated with antidepressant medication.[83] In contrast, transgenic mice with reduced BDNF levels and transgenic mice with reduced signalling of the BDNF receptor TrkB were both resistant to the effects of antidepressants in the forced swim test model of depression.[84] These studies indicate that TrkB signalling may be required for an antidepressant effect, and that antidepressant-induced upregulation of BDNF may be a common mechanism of action.

Exercise has been shown to increase BDNF,[77,85] increase neurogenesis[77,85] and act as an antidepressant in both the learned helplessness and forced swim tests.[86,87] In a similar fashion to the chemical antidepressants, it may be that the exercise produces its antidepressant effects by upregulating cell proliferation and BDNF. In support of this, it has been shown that although both exercise and antidepressants prevent swim stress-induced decreases in BDNF, the combination of both treatments potentiates this effect.[86,87]

BDNF is also one of the targets of the cyclic adenosine monophosphate (cAMP) signal transduction cascade and the transcription factor cAMP response element binding protein (CREB). Both cAMP and CREB have been implicated in the antidepressant response with respect to behaviour and cell proliferation.[65] The cAMP pathway is upregulated after long-term antidepressant treatment, and one hypothesis is that this upregulation signals BDNF and CREB to positively affect neuronal survival and neural plasticity in order to produce antidepressant effects.[5] However, Conti et al[88] have reported that CREB-deficient mutant mice are still able to display the normal behavioural responses to antidepressants. This indicates that there is also a CREB-independent pathway for some of the pharmacologic actions of antidepressants.

Taken together, it may be that one way the antidepressants work is by neurotrophic-mediated changes in neurogenesis and behaviour. Future studies will determine whether the neurotrophic-induced increases in cell proliferation are necessary for the therapeutic effects.

Role of glial cells in antidepressant action

One hypothesis is that the antidepressants work through glial cells, in addition to neurons, to exert their effects. Although most BrdU-positive cells mature into neurons, a small percentage of glial cells are also born. After antidepressant treatment, an increased number of cells are born, and the same percentage differentiates into glia.[16] Therefore, a net increase in glial cells is produced after antidepressant treatment, although it is a much smaller amount compared with the net increase in the number of neurons. The Ca^{2+}-binding glial pro-

tein S100B has been shown to play a role in regulating cellular function and structural support.[89] Recently, it has been shown that S100B affects long-term potentiation, as well as memory and learning, indicating that it may also be important in mediating synaptic plasticity.[90]

A number of studies point to a possible role of glial cells and S100B in mediating antidepressant-induced neurogenesis. Long-term fluoxetine treatment increases S100B protein in the rat hippocampus.[91] Clinically, postmortem studies show decreased numbers of both glial and neuronal cells, indicating that not only neurons but also glia are downregulated in the pathophysiology of depression.[66–68] Finally, in-vitro studies demonstrate that astrocytes may have an active role in directing neurogenesis.[92] Further experiments will be needed to determine what role this relatively small population of cells plays in the mechanism of antidepressant-induced neurogenesis.

Correlation or causation?

One of the main difficulties in this field has been proving the connection between antidepressants and neurogenesis. Most studies of antidepressant effects report correlations between the experimental manipulations and changes in cell proliferation and neurogenesis. Recently, however, a number of investigators have sought to prevent cell proliferation in order to determine the role of the proliferating cells in the maintenance of antidepressant-induced behaviour.

Santarelli et al[3] have demonstrated that the newly born hippocampal cells play a role in antidepressant-induced behaviour. In this study, mice were given x-ray irradiation, which prevents cell proliferation. Irradiated mice were then given antidepressant drugs and tested in 1 of 2 behavioural paradigms. The antidepressant-treated irradiated mice did not display the expected antidepressant response on either test. Although the irradiation methodology has its limitations, this was the first evidence that hippocampal cell proliferation is necessary for antidepressant action. The authors concluded that the cessation of neuronal cell proliferation and neurogenesis is responsible for the lack of effect of the antidepressants.

Shors et al,[93] in an earlier study, had administered an agent that produced a global cessation of cell proliferation and found deficits in hippocampus-associated learning. These results indicate that these newly born cells serve an important function for learning, although these same authors have recently reported that not all hippocampus-dependent learning is dependent on neurogenesis.[94] The link between neurogenesis and learning is of interest given that depressed patients also exhibit cognitive deficits.

Studies such as these, or those that use transgenic animals to produce conditional ablation of immature or newly proliferating neurons, will be necessary to prove the casual link between depression, antidepressant action and neurogenesis.

Conclusion

An increasing body of evidence from both clinical and basic research makes a compelling case for antidepressants working to reverse the negative effects of stress on hippocampal function. Future research should focus on the use of animal models of depression to more fully understand how the induction of cell proliferation and neurogenesis relates to antidepressant-induced behaviour. On a cellular and molecular level, understanding the mechanisms regulating hippocampal cell proliferation and neurogenesis in the stressed and antidepressant-treated animal will be critical.

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