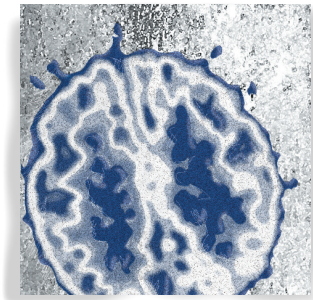


Stem cell approaches in psychiatry—challenges and opportunities

Jens Benninghoff, MD



Exploring stem cells is a fascinating task, especially in a discipline where the use of stem cells seems far-fetched at first glance, as is the case in psychiatry. In this article we would like to provide a brief overview of the current situation in relation to the treatment of mental diseases. For reasons that we will explain, this review will focus on affective disorders. The following section will give a more detailed account of stem-cell biology, including current basic science approaches presenting in-vivo and in-vitro techniques. The final part will then look into future perspectives of using these stem cells to cure mental illnesses, and discuss the related challenges and opportunities.

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Regenerative medicine appears to be on the brink of a bonanza in terms of new treatment options, ie, stem cell-based therapy. Up to now, stem cell-based interventions (SCBI) have still been in an immature state. Only a few trials are currently under way, and are so far mostly in a preclinical phase. Current focuses include Duchenne's disease, Parkinson's disease, and Alzheimer's disease.¹ The major concept of all these experiments is to create a treatment scheme similar to that in bone-marrow diseases where hematopoietic stem cells are regularly used as a cure for certain types of leukemia—in this case, the issue of the appropriate stem cell type used has been solved. For SCBI in neurodegenerative disease there is an ongoing debate regarding which cell type might be suitable for transplantation—embryonic versus fetal versus adult stem cells. Furthermore, the question of stem-cell homing needs to be addressed, since one may not need to transplant the cells by neurosurgical procedures. Instead, it could be sufficient to inject these cells into the cubital vein only,² since the plasticity of these cells enables them to find the niche where they are needed—even within the central nervous system (CNS).

Apart from technical aspects, ethical problems arise. Even without touching on the debate of using human embryonic stem cells, there is plenty of groundwork for bioethicists to do.

When the ethical and technical issues have been resolved, we may proceed from neurodegenerative to psychiatric illnesses such as affective disorders and schizophrenia.

We still face a substantial lack of proof as to whether these psychoses are the cause or the correlate of disturbed adult neurogenesis.³ If so, we may consider these severe illnesses as being neurodegenerative, as there is some compelling data for this, at least in the field of

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Selected abbreviations and acronyms

5-HT	<i>serotonin</i>
BDNF	<i>brain-derived neurotrophic factor</i>
CREB	<i>cAMP response element binding protein</i>
ES cells	<i>embryonic stem cells</i>
SCBI	<i>stem cell-based interventions</i>

depression. There may be some clinical trials of grafting stem cells, in a long and cumbersome process, into the brains of diseased patients. In our opinion, this will only be the case for very severe cases of depression, after having tried nearly all the available medication options and unsuccessful electroconvulsive therapy (ECT).

Past and current status

In the past, psychiatric diseases have been treated pharmacologically with broad-profile medication—the so-called “shotgun method.” In the same way that a shotgun fires many pellets at once, psychiatric medication can impact on many different neurotransmitter systems. Due to this profile, many of these drugs, such as tricyclic antidepressants (TCAs) or first-generation antipsychotics (FGAs) caused severe undesirable side effects, which were held responsible for poor compliance and discontinuation of the prescribed medication.

During the last two decades, new drugs have surfaced with fewer shotgun side effects because of their particular pharmacodynamic design targeted against one single and very specific molecule. In this context, selective serotonin reuptake inhibitors (SSRIs) should be mentioned here as an example of an antidepressant agent acting solely on one neurotransmitter system and within the serotonergic system on one distinct transporter molecule. The same holds true for second-generation antipsychotics (SGAs) displaying only few side effects due to less rigid inactivation of dopamine receptor type 2 (DR2) and therefore fewer extrapyramidal motor symptoms recalling parkinsonism.

Nevertheless, we are still struggling with inefficacious medication, since only about one third of antidepressant agents work in a given patient, meaning that one has to try on average three different medications in order to alleviate this patient’s symptoms. For schizophrenia the same holds true. Sometimes, the individual situation seems even worse than in the field of affective disorders.

Some stem cell basics

As mentioned above, this article emphasizes the link between disturbed adult neurogenesis (AN) and affective disorder. The case seems to be much more evident here than in schizophrenia, although decreased neural stem cell proliferation in the dentate gyrus (DG) of the hippocampus has been demonstrated in postmortem human brain from schizophrenic patients.⁴

Stem cells can be characterized by two fundamental qualities: first, they have the capacity for unlimited self-renewal, and second, they can produce at least one type of highly differentiated descendants.⁵ This particular cell division is termed “asymmetrical”: in general, each stem cell division gives rise to one stem and one committed somatic daughter cell.⁶ Stem cells are single cells that, once developed, self-renew for the lifetime of the organism. These stem cells should be distinguished from transient progenitor cells, which have a limited self-renewal lifespan.⁷ Some steps earlier during the embryonic period, cells become gradually restricted to distinct pathways of differentiation. This process includes modification of their developmental potential; they become pluripotent (“many, several”). The major difference between totipotency and pluripotency is that an embryonic stem cell (ES cell) which is by definition “pluripotent,” can only form cells which constitute the embryo itself but not the placenta. Early ES cells can be taken from the embryo and grown in vitro. When retransferred into the embryo, these cells can still generate all tissues, including the germ line.⁸ ES cells also play a central role in the generation of transgenic animals such as knockout mice.

Pluripotency of stem cells becomes progressively restricted and they become multipotent. Multipotent stem cells in the brain ultimately give rise to all different types of neuronal and non-neuronal cells in the central nervous system. Presumably they have lost the ability to produce cells of ento- and mesodermal origin. Because they are capable of generating the entire progeny of a given tissue, some investigators have termed these multipotent stem cells “progenitor cells.” Moreover, there is controversy as to whether neural stem cells can actually remain viable during the entire lifespan.⁹

Adult forebrain neural stem cells were discovered in 1992¹⁰ in the adult remnant of the embryonic brain germinal zone surrounding the lateral ventricle. Evidence for their participation in repopulating the adult lateral ventricular subependyma following irradiation¹¹ led to the

hypothesis that neural stem cells also exist after the embryonic and fetal period, similarly to hematopoietic stem cells. Hematopoietic stem cells in adult animals can restore different blood cell types. For a long time it has been thought that once a cell had been programmed to produce a particular tissue, its fate was sealed and it could not reprogram itself to form another tissue. Contrarily to this view, reactivation of dormant genetic programs appears to work under certain circumstances. Intriguingly, stem cells from brains of adult mice have been shown to possess the potential to become functional blood cells.¹² Similar results for the inverse case were reported, ie, multipotential mesenchymal cells transformed into neural cells—astrocytes in this case.¹³

According to Fred H. Gage, the term “neural stem cell” should therefore be used with caution to describe cells that “a) generate neural tissue, b) are capable of self-renewal, and c) give rise to cells other than themselves through asymmetric cell division.”¹⁴ In fact, when the issue of lineage specificity of adult neural stem and progenitor cells is considered, there is abundant reason for caution regarding the term neural stem cell. The ability of bone marrow stem cells to generate astrocytes,¹⁵ the finding that astrocyte-like cells in the subependyma are neural stem cells,^{15,16} and that oligodendrocyte-precursors contribute neural stem cell-like cells¹⁷—all these findings soften the theoretical distinction between stem and progenitor cells. All this makes it really difficult to decide which type of stem cells one should use for transplantation. Even during the process of grafting stem cells they can still start to differentiate and become more restricted progenitors due to cell-cell contacts or influence of adhesion inside the syringe.

Further, neural stem cell research suffers dearly from the lack of an antibody specifically identifying neural stem cells. Detecting stem cells ad hoc and not ex post remains a problem. Putative stem cells need to differentiate into their derivative neural cell subpopulations before one can positively identify them as regular neural stem cells. In the past, efforts to generate antibodies against adult stem cells did not prove sustainability.^{18,19} There are certain advantages of adult over embryonic stem cells in that the former may be easier to manage. ES cells tend to differentiate spontaneously into all kinds of specified tissue. For example, when injected subcutaneously into immunocompromised mice they grow into teratomas, tumors consisting of numerous cell types ranging from gut to skin. Before applying these cells in humans, gen-

eration of the desired cell types should therefore be ensured without undesired side effects or unwanted cell populations, respectively. Here, it seems that adult stem cells are better behaved, since they do not differentiate spontaneously.

Instead this can be induced by applying appropriate growth factors. However, adult stem cells have a different drawback, in that they seem to lose their ability to divide and differentiate after some time in culture. Maybe in the end ethical considerations will also convince the scientific community to follow the adult stem cell rather than the ES cell track. Compared with embryonic or fetal stem cells, adult stem cells pose fewer ethical problems because they can be obtained from sources other than embryos or aborted fetuses.²⁰ Even post-mortem human tissue can yield neural stem cells.²¹ In consensus with Frank E. Young²² the public, as well as governmental authorities, should enter the process of unbiased dialogue, in order to establish the principles according to which research needs to be conducted.

As of now, we should consider the human species an appropriate source for SCBI. Having said this, we should take into account possible chimerae of animals with human cells in their brains, and above all with possible human behavior.²³ Another issue to be ruled out is to prevent striking behavioral traits after SCBI. In Parkinson's disease patients it has been shown that after L-Dopa treatment some patients responded with pathological gambling,²⁴ since dopamine sustains the reward system. One could easily imagine a scenario like this in a patient after SCBI. In this case, it might not be as easy to lower the dopamine production as it is with cessation of the medication.

As mentioned above, grafting hematopoietic stem cells has already become a conventional clinical tool in the treatment of certain types of leukemia. Currently it can only be hypothesized that transplantation of neural stem cells has potential for treating brain disease.²⁵

Although all these obstacles do exist, the main target for the research on neural stem cells must be to restore regular neural function in areas where cells have died or lost their physiologic behavior. Clinicians are eager, for example, to transplant NSCs into patients suffering from Parkinson's disease,²⁶ multiple sclerosis, or spinal cord injuries, although it is not clear so far which is the appropriate cell to transplant—the CNS neural stem cells, the actual neurons, or intermediate progenitors between the two. Thus, in neurodegenerative diseases it is important to first determine the rules of transplantation of stem,

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progenitor, and mature cells, as well as to determine the sites into which the transplants must be located. In Parkinson's disease we do not know whether cells should be placed into the substantia nigra, or striatum, or both. So we continue wondering which particular region of the abovementioned brain areas should be chosen—eg, the ventral part or the dorsal putamen in only one hemisphere or both. The variability in surgical methods between centers which perform stem cell transplantation makes it very difficult to assess the results of this procedure.²⁶ Still, if we get to the point where all these issues are resolved, we will have to find better ways to secure the long-term survival of the grafted cells.

So far, the *de novo* generated dopaminergic neurons have only a limited lifespan in animal studies.²⁷ Finally, the source of the cells should be clarified, since we need huge amounts of human fetal tissue for transplantation. Although there has been much progress achieved over the last few years, especially in long-term proliferation and dopaminergic differentiation of progenitor cells,²⁸ we still have to invest much effort in finding alternative methods, such as *in vivo* mobilization of dormant neural stem cells, which seems to be a more useful concept.

Pathophysiologic models of depression

In the light of recent findings such as loss of total cell volume in certain brain areas observed in depressed patients (see below), we can heretically define depression as a “neurodegenerative” disease. As we will discuss, this particular illness does not necessarily require stem cell transplantation. Instead, it may be possible to replenish missing neurons by regulating the microenvironment surrounding the putative sites of neural stem cells where neurogenesis takes place. Lessons from recent findings in pathophysiology might open up a broad research avenue with the ultimate goal of treating depression.

In a WHO survey, depression and manic-depressive illness will still rank among the top 10 causes for death in the year 2010. Certainly not all forms of depression eventually lead to death by suicide, but even milder courses of this illness may lead to severe incapacitation of millions of people worldwide. In particular, it impairs reintegration into their familiar social environment and into the working process. Accumulating evidence from neurobiology suggests that distinct biochemical processes are derailed in a large number of depressed subjects. Cellular and molecular adjustments following stress seem to play

key roles in onset and propagation of mood disorders. To most of us, stress is a beneficial response of our neuronal systems to acute challenges of the exterior world. To put it simply, it is more beneficial for a rabbit to become stressed and flee when it sees a fox approaching. However, severe or repeated stress can lead to detrimental effects on regular neural function.²⁹

Neural plasticity is a term which involves interneuronal communication and adaptation in order to give the appropriate responses to stress or aversive stimuli. One example of such a response could be changes in neurogenesis—the generation of new neurons. Dysfunction of adequate regulatory processing due to severe or chronic stress is capable of disturbing neural plasticity.

Medical treatment with antidepressant agents may bring back regular function of neural systems by influencing neural plasticity: antidepressants require long-term administration, while blockade of the reuptake of serotonin (5-HT) and/or norepinephrine as their most common and initial mode of action is fairly rapid. There is a process whereby neurons can adapt to and regain plasticity while the local biochemical environment is changing due to the application of antidepressants. Thus, the long-term mode of action of antidepressant medication seems much more dynamic and complex than just up- or downregulation of synaptic levels of monoamines.

The role of the hippocampus

The hippocampus is a well-characterized brain structure. In 1886 C. Golgi stained hippocampal neurons with his novel silver impregnation technique, which became known as the Golgi procedure. Since then a great number of neuropsychiatric phenomena have been studied in the hippocampal formation.

The relatively simple organization—pyramidal neurons in the hippocampus proper and the granule cells of the dentate gyrus are arranged in single, densely packed cell layers—is one of the major reasons why the hippocampus has frequently been used as a cytoarchitectural model of the cortex. Recent findings in volumetric neuroimaging studies make a strong case that biochemical changes in the brain carry morphological sequelae. So far we have learned that gray matter volumes are diminished in depressed patients and in post-traumatic stress disorder patients in the medial and orbital prefrontal cortex, the mesiotemporal cortex, and the ventral striatum, and are accompanied by an enlargement of the third and the

lateral ventricles.³⁰⁻³³ Hippocampal gray matter volume is reduced strikingly in depressed patients.^{34,35}

Additional postmortem brain studies underpin the above-mentioned results. According to Vincent et al³⁶ there is a layer-specific reduction of interneurons in the anterior cingulate cortex. Significant reduction in numbers of non-pyramidal neurons in the CA2 area of hippocampus was reported in postmortem studies of bipolar disorder.³⁷ Also in regions other than hippocampus, there may be a decline in brain region volume and total cell number.^{38,39}

Elevation of cortisol levels in the elderly correlates with reduced hippocampal volume, and is associated with memory deficits.⁴⁰

Patients with depression have a functional deficit of the hypothalamic-pituitary-adrenal (HPA) axis.⁴¹ Hippocampal neurons are reported to be damaged by exposure to stress or activation of the HPA axis and elevation of glucocorticoids. Taken together, this overview of morphologic evidence strongly supports a functional link between changes at the molecular levels and morphology. The task of future research could be to develop strategies allowing the diseased hippocampus or other affected brain structures to regain regular morphology and function. Focusing on neurogenesis—which is defined as a series of events including proliferation of a neural precursor or stem cell that results in appearance of a new neuron⁴²—may be a systematic as well as pragmatic way to proceed.

There is a growing body of evidence for the phenomenon of neurogenesis in humans.¹⁵ Localization of pluripotent progenitor cells and thus neurogenesis appears to be restricted to certain brain regions, in particular, the subventricular zone (SVZ) and the subgranular layer of the dentate gyrus of the hippocampus.⁴² Neurogenesis in the adult mammalian brain is regulated by genetic and environmental factors⁴³⁻⁴⁵—all leading to the exciting possibility of pharmacological regulation of neurogenesis in the adult brain, and eventually of the disease-related pathophysiological changes.

One of the mainstay therapies in the treatment of recurrent mood disorders, lithium, ranks among such pharmacologic candidates. Lithium increases the levels of the antiapoptotic protein bcl-2.^{46,47} We now know that besides its role in cell cycle control, bcl-2 functions as a neurotrophic factor, since bcl-2 promotes axon regeneration as well as neurite and axonal outgrowth.⁴⁸ In general, neurotrophic factor signaling is mediated both by the phosphatidylinositol-3-kinase pathway and activation of the MAP (mitogen-activated kinase) cascade.^{49,50}

Activation of MAP cascade augments bcl-2 expression. This is very likely to involve the cAMP responsive element binding protein (CREB).⁵¹

CREB is attractive to many researchers because it appears in some way required for long-term memory.⁵² CREB may increase the integrity and functional plasticity of granule cell neurons assuming that CREB is a critical determinant of neural plasticity as well as cell survival. One putative gene target of CREB—and thus of chronic antidepressant treatment—is brain-derived neurotrophic factor (BDNF). There is a functional cAMP responsive element in the exon III promoter of the BDNF gene.⁵³ In the light of this, it is not surprising that local infusion of BDNF in the hippocampus produces an antidepressant effect.⁵⁴

In vitro, activation of the cAMP system upregulates BDNF expression in hippocampal cells.^{55,56} Additionally, BDNF expression effects neuronal depolarization and activation of voltage-dependent calcium channels. These alterations at the synaptic level underlie the influence of BDNF on long-term potentiation.⁵⁷ This underscores the central role of BDNF in neurogenesis considering the pivotal role attributed to BDNF in lineage differentiation of neural stem cells.

Another key player in the pathophysiology and treatment of depression, the biogenic amine 5-HT, should not be neglected, since 5-HT is one of the most extensively studied neurotransmitters of the central nervous system. Moreover, novel findings indicate that 5-HT is particularly relevant to neurogenesis in the hippocampus (*Figure 1*), because in adult rats it has been shown that decreased 5-HT lowers the rate of neurogenesis in the dentate gyrus of hippocampus.⁵⁸ Historically, 5-HT was first described as a serum component augmenting

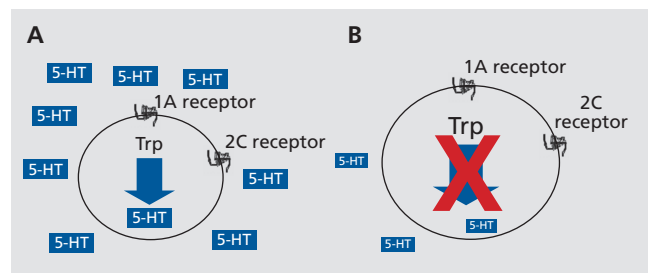


Figure 1. A) Putative model of the serotonergic (5-HT) machinery in adult neural stem cells. Tryptophan hydroxylases (TPH) produces 5-HT, which controls its own metabolism mainly via 5-HT1A and 5-HT2C receptors. B) TPH inactivation leads to less 5-HT production—model proposed by the author based on own experimental data (Benninghoff et al, unpublished data).

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smooth muscle contraction.⁵⁹ In such non-neural systems, 5-HT has been a potent mitogen.^{60,61}

In the brain, 5-HT is among the most widely distributed neurotransmitters. All serotonergic fibers originate in the brain stem raphe nuclei. By way of extensive synaptic connections of the serotonergic fibers, 5-HT contributes to many physiologic functions such as endocrine and circadian rhythms, food intake, sleep, reproductive activity, and motor function, as well as cognition, mood, and anxiety.⁶² In the brain we currently know of 16 different cloned receptor types and subtypes, but it can be expected that their number will grow even further in the near future. In contrast to the multitude of 5-HT receptors, there is only a single 5-HT transporter (5-HTT) responsible for the reuptake of 5-HT into serotonergic neurons after its release into the synaptic cleft. As our own studies have shown, 5-HTT does not have a large impact on neurogenesis.⁶³

A possible role for 5-HT as direct mediator of granule cell generation is currently discussed, since elevated 5-HT levels in the hippocampus increase the rate of proliferation of granule cell precursors.⁶⁴ Epidermal growth factor (EGF) is believed to exert an essential function on the generation and maintenance of neural stem cells. It is therefore not surprising that in non-neural systems, EGF and 5-HT can augment the rate of cell proliferation in a synergistic manner.⁶⁵ BDNF again seems involved in mediating the effects of 5-HT. Thus, chronic administration of 5-HT-selective reuptake inhibitors, clinically used as antidepressants, leads to upregulation of BDNF mRNA.⁶⁶

As already mentioned above, 5-HT exerts its action through a large family of receptors in the periphery and throughout the CNS.⁶² A possible role for the 5-HT_{1A} receptor in the modulation of anxiety and depression, as well as in the mode of action of anxiolytic and antidepressant drugs, has been suspected for many years.⁶⁶ 5-HT_{1A} receptors operate both as somatodendritic autore-

ceptors and postsynaptic receptors. Research regarding 5-HT_{1A} receptor has shown⁶⁷ that the effect of antidepressants upregulating extracellular serotonin levels worked via the 5-HT_{1A} receptor subtype, thus opening a link between our in vitro system, neurogenesis, and clinical relevance in terms of affective disorders.

Although the serotonin hypothesis of depression⁶⁸ is very attractive in this regard, it should not be omitted here that there are additional compelling findings dealing with other neurotransmitting systems, eg, supporting cholinergic mechanisms.⁶⁹ Riederer et al⁷⁰ came to the conclusion that a brain area-specific imbalance of neurotransmitters leads to the clinically different manifestations of depression. The loss of regional interneuronal homeostasis must not necessarily affect huge brain areas; it might be only limited to certain small and circumscribed regions in the brain. As a consequence, clinicians should be able to choose the pharmacologically appropriate medication for the affected brain region.⁷¹

Concluding remarks

Stem-cell maintenance and generation take place in a distinct microenvironment where appropriate external signals can best exert their regulatory function on these cells. Signals provided by neural growth factors are responsible for neural stem-cell growth. Since components of regular stem-cell maintenance like BDNF are also implicated in mechanistic models characteristic of mood disorders, they thus offer new targets for pharmacologic intervention in neuropsychiatric disease. More thorough knowledge about this complex connection may help us render antidepressant treatment more efficient and reduce the undesirable side effects that impair patient compliance. So far there is no stem-cell-based approach really on the horizon for treating depression or any other psychosis. □

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Acercamiento a las células madre en psiquiatría: desafíos y oportunidades

La exploración de las células madre es una tarea fascinante, especialmente en una disciplina donde el empleo de ellas parece exagerado a primera vista, como es el caso de la psiquiatría. En este artículo se quiere entregar una breve panorámica de la situación actual en relación con el tratamiento de las enfermedades mentales. Por razones que serán explicadas, esta revisión se centrará en los trastornos afectivos. La sección siguiente dará cuenta más detallada de la biología de las células madre, incluyendo las propuestas actuales de la ciencia básica que se presentan en técnicas tanto in-vivo como in-vitro. La parte final se orientará hacia las perspectivas futuras del empleo de estas células madre para la cura de las enfermedades mentales, y se discutirán los desafíos y oportunidades relacionadas con ellas.

Approches des cellules souches en psychiatrie : défis et perspectives

Explorer les cellules souches est passionnant, surtout dans un domaine comme la psychiatrie où, au premier abord, leur utilisation semble incongrue. Cet article souhaite offrir un aperçu de la situation actuelle en rapport avec le traitement des maladies mentales et, pour des raisons qui seront données ultérieurement, il ne traitera que des troubles de l'humeur. La première partie détaillera la biologie des cellules souches, y compris les approches scientifiques actuelles des techniques in vivo et in vitro. Ensuite, les perspectives d'utilisation des cellules souches dans le traitement des maladies mentales seront présentées et les défis et opportunités qui y sont liés seront discutés.

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