# The serotonergic system in health and disease

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**Subtitle: The role of serotonin in adult hippocampal neurogenesis**

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**Abstract**

Serotonin is probably best known for its role in conveying a sense of contentedness and happiness. It is one of the most unique and pharmacologically complex monoamines in both the peripheral and central nervous system (CNS). Serotonin has become in focus of interest for the treatment of depression with multiple serotonin-mimetic and modulators of adult neurogenesis used clinically. Here we will take a broad view of serotonin from development to its physiological role as a neurotransmitter and its contribution to homeostasis of the adult rodent hippocampus. This chapter reflects the most significant findings on cellular and molecular mechanisms from neuroscientists in the field over the last two decades. We illustrate the action of serotonin by highlighting basic receptor targeting studies, and how receptors impact brain function. We give an overview of recent genetically modified mouse models that differ in serotonin availability and focus on the role of the monoamine in antidepressant response. We conclude with a synthesis of the most recent data surrounding the role of serotonin in activity and hippocampal neurogenesis. This synopsis sheds light on the mechanisms and potential therapeutic model by which serotonin plays a critical role in the maintenance of mood.

**Introduction**

Our knowledge of the biology of serotonin (5-hydroxytryptamine, 5-HT) and its function in the peripheral and CNS has been rapidly growing. This is likely due to the cloning of the multiple receptor subtypes that mediate serotonin signaling and the advent of new technologies to selectively perform gain- or loss-of-function studies. In this review we focus on serotonin activity in the rodent brain, and particularly concentrate on the role of serotonin in adult hippocampal neurogenesis. The discovery of newly generated neurons throughout life (Altman and Das, 1965, Kaplan and Hinds, 1977) has not only altered the perception of the general public that the brain lacks the capacity for neuron replacement but also opens novel fields of neuroscience research. Serotonin contributes to this fascination as one of the crucial signals in the neurogenic niche microenvironment together with other growth factors, hormones, or neurotransmitters that regulate cell proliferation and differentiation.

Neurogenic niches are only a few spatially restricted regions consisting of neural stem cells that retain fate plasticity and can respond to environmental stimuli (Kempermann et al., 1997, van Praag et al., 1999, Doetsch and Hen, 2005). These are the subventricular zone (SVZ), where new neurons contribute to encoding olfactory information, and the subgranular zone (SGZ) of the dentate gyrus (DG). In the adult DG, neurogenesis comprises six developmental steps, where radial glia-like stem cells (type-1, 1$^{st}$) give rise to proliferating amplified progenitor cells (type-2a/b and type-3, 2$^{nd}$ to 4$^{th}$) that become immature (5$^{th}$) and mature (6$^{th}$) neurons (Kempermann et al., 2004, Hodge et al., 2008) (Figure 1A). The common method to examine neurogenesis includes proliferation and differentiation of precursor cells that can be detected by incorporation of the thymidine analog bromodeoxyuridine (BrdU), and specific marker expression such as Sox2 (type-1 and type-2) or DCX (doublecortin) that characterizes transiently amplified progenitor cells of the neuronal lineage (type-2b/3). While proliferation is determined two to 24 hours after, survival of newborn neurons is analyzed several times post-injection of BrdU; the time newborn cells get structurally and functionally integrated into the network is within three to seven weeks (Lie et al., 2004). The young adult rat DG generates approximately 9,000 cells/day (6% of the total granule cell population per month) that drastically declines with aging (Kuhn et al., 1996, Cameron and McKay, 2001).

Serotonin plays a role in many homeostatic systems as evidenced by the results showing that deregulation of the serotonin system leads to neurogenic decline, changes in appetite, and mood disorders. Whether neurogenic decline is causative or associative with these pathologies remains to be determined. In the case of depression, chronic manipulation of serotonin by agents that inhibit serotonin reuptake, leads to clinical improvement associated with a slow, temporal increase in adult hippocampal neurogenesis (Malberg et al., 2000, Santarelli et al., 2003). While the mechanism of serotonin action in the hippocampus is just being illuminated, there is considerably less known regarding its function in SVZ neurogenesis (Banasr et al., 2004, Ohira and Miyakawa, 2011).

The first part of this chapter will summarize studies on robust pharmacological depletion or enhancement of serotonin levels that affect granule cells in the DG. We will discuss the modulator role of several 5-HT receptors and their contribution to homeostasis of adult neurogenesis. As we will see, serotonin exerts a pro-neurogenic effect that is mediated through a complex network of receptors, many of which remain to be tested. We focus on the receptors addressed the most: 5-HT1A, 5-HT2A and 2C, and summarize the key findings. The review will continue with a variety of recently generated animal models that provide controlled regulation of serotonin supply and have been used to characterize alterations in baseline proliferation and survival of newborn cells. It is important to define serotonin's essential roles in brain function and behavior, as it is equally important to

discover the regulators and interactive pathways by which serotonin mediates brain function. In this regard, we have recently tested serotonin action in a loss-of-function model in which the availability of brain-derived serotonin is selectively depleted (Alenina et al., 2009, Klempin et al., 2013). These data together with others from genetically modified mouse models shed a distinct light on the mechanisms proposed in earlier studies to be reviewed here. We will also consider the anticipated role of serotonin in the antidepressant response and increased neurogenesis and thus address and review the monoamine/neurotrophin hypothesis. Finally we will point toward new directions for future research and discuss the promise of uncovering mechanisms where serotonin may be manipulated to affect memory and mood disorders.

*The effect of pharmacological serotonin depletion or enhancement on baseline neurogenesis*

Serotonin is synthesized in neurons of the brain stem raphe nuclei (reviewed in (Gaspar et al., 2003)) from the amino acid tryptophan by a short metabolic pathway, consisting of the neuron-specific enzyme tryptophan hydroxylase (TPH) 2 (Walther et al., 2003). Once serotonin is synthesized it is packed into synaptic vesicles by the vesicular monoamine transporter (VMAT) 2. Extracellular levels of serotonin are regulated by its re-uptake into the presynaptic cell through its specific (serotonin) transporter (SERT). The transcription factors Lmx1b and Pet1 are expressed in serotonergic neurons in the raphe nuclei during development. Pet1 directly controls the expression of the genes encoding TPH2, 5-HT1A receptor, and SERT (Liu et al., 2010) by binding a conserved *cis*-regulatory element in these genes (Hendricks et al., 1999).

Serotonergic fibers project throughout the brain and into the DG of the hippocampus where they synapse with granule cells and interneurons. Here, serotonin is an important intrinsic soluble factor that promotes neuronal development (Brezun and Daszuta, 2000, Radley and Jacobs, 2002, Santarelli et al., 2003, Banasr et al., 2004); but data on whether this stimulation primarily affects cell proliferation or survival of newly generated neurons are contradictory. First evidence of serotonin's modulator role in the adult DG originates from pharmacological manipulation studies based on lesion of serotonergic neurons in the raphe (by injection of 5,7-Dihydroxytryptamine, DHT) (Brezun and Daszuta, 2000, Jha et al., 2006) or inhibition of serotonin synthesis (by para-chlorophenylalanine, PCPA) (Huang and Herbert, 2005b, Jha et al., 2006). While the first method robustly destroys fibers and possibly cells, PCPA treatment inhibits the synthesizing enzyme TPH. Yet, both methods have been shown to decrease hippocampal serotonin levels and adult neurogenesis (Brezun and Daszuta, 2000, Jha et al., 2006). In turn, raphe grafts can restore serotonergic control and the number of proliferating precursor cells and immature neurons in the DG (Brezun and Daszuta, 2000). Serotonin depletion also leads to a decrease in

dendritic spine density of granule cells (Yan et al., 1997). However, controversial data exist showing that PCPA treatment, but not the neurotoxin 5,7-DHT (Huang and Herbert, 2005b) might decrease the number of precursor cells at 1 day and 4 weeks later (Jha et al., 2006), while chronic PCPA treatment also leads to increased survival of precursor cells in the DG of wild type animals mimicking models with serotonin deficiency (Diaz et al., 2013). On balance, data from these studies reveal that serotonin depletion or a decline in serotonin synthesis has long-term regulatory effects on adult hippocampal neurogenesis, however with noticeable contradictive effects.

Further evidence of serotonin's modulator role on generating new neurons in the adult brain has been achieved by studies on selective serotonin reuptake inhibitors (SSRIs) (Malberg and Duman, 2003, Santarelli et al., 2003, Encinas et al., 2006, Wang et al., 2008, Klempin et al., 2010). SSRIs mode of action is an immediate increase in the synaptic availability of serotonin with the acute administration facilitating serotonergic transmission and transient desensitization of 5-HT1A autoreceptors. Chronic treatment of this class of antidepressants induces re-establishment of the serotonin system by long-term downregulation of SERT and 5-HT1A autoreceptor activities (Blier and Ward, 2003, Descarries and Riad, 2012). Chronic, but not acute, treatment with fluoxetine (trade name 'Prozac') also leads to increased hippocampal neurogenesis, e.g. increased cell proliferation and number of newborn neurons (Malberg et al., 2000, Santarelli et al., 2003, Encinas et al., 2006, Klempin et al., 2010). Later we will review the characteristic delay observed in serotonin-based drug efficiency, and return to the mechanism underlying the antidepressant hypotheses from animal studies and a clinical perspective.

*Pharmacological 5-HT receptor targeting studies and baseline neurogenesis*
Serotonin's role in the hippocampus has been extensively discussed relying on studies using pharmacological depletion or receptor targeting. We have recently shown that 5-HT1 and 2 receptor subtypes control both acute and chronic effects of serotonin release that modulate different developmental steps in adult neurogenesis (Klempin et al., 2010). Serotonergic targets within the DG express a variety of 5-HT receptors that in turn dictate the response from efferent activity (Brezun and Daszuta, 2000, Klempin et al., 2010, Diaz et al., 2013). Their expression pattern varies with 5-HT1A receptor immunoreactivity found on type-1 to type-2b cells in the SGZ as well as on interneurons. For 5-HT2A receptors, labeling revealed a dense staining in the hilus, whereas 5-HT2B (Diaz et al., 2013) and 5-HT2C receptors mostly mark adult granule cells (Klempin et al., 2010). 5-HT3 receptor mRNA expression was seen in hilar interneurons (Tecott et al., 1993) that was confirmed by *in situ* hybridization (Diaz et al., 2013). We hypothesize that the distinct actions of receptor subtypes could mediate the homeostasis of serotonin signaling in the

hippocampus. In the following, we are reviewing studies on receptor targeting by manipulation with a variety of agonist and antagonists.

A predominant mediator of serotonin action is the 5-HT1A receptor subtype as seen in neurosphere cultures and selective receptor targeting studies in rat and mouse models. Neurosphere assays are used to investigate neural stem cells *in vitro* and to define their stemness and multipotency. Under proliferation conditions, neural stem cells produce both, TPH and serotonin, and express 5-HT1A receptors (Benninghoff et al., 2010, Klempin et al., 2010). Self-renewal and proliferation is decreased by 5-HT1A blockage or PCPA treatment that is rescued by exogenous serotonin. Acute targeting of 5-HT1A postsynaptic receptors with selective antagonists *in vivo* has been shown to reduce the number of newly generated cells in the DG (Radley and Jacobs, 2002) whereas acute stimulation had the opposite effect (Banasr et al., 2004, Klempin et al., 2010). Furthermore, chronic activation also increases proliferation, while prolonged inhibition of 5-HT1A receptors results in a decrease in net neurogenesis (Banasr et al., 2004, Klempin et al., 2010). Combined treatment with PCPA (or following 5,7-DHT, respectively) and activation of 5-HT1A postsynaptic receptors increases precursor cell proliferation in the DG (Huang and Herbert, 2005a). Activating 5-HT1B receptor subtypes also seem to mediate the proliferative effect as recovery was observed following PCPA treatment in rats (Banasr et al., 2004). The decrease in dendritic length and loss of spine density observed after serotonin depletion is due to decreased 5-HT1A receptor activation as the same effects have been seen following 5-HT1A antagonist treatment (Yan et al., 1997). The effect was reversed by stimulation with a selective 5-HT1A agonist. Overall synaptogenesis seems to be mediated by 5-HT1A receptor signaling (Mogha et al., 2012). Interestingly, pharmacological manipulation of the 5-HT2 receptor family reveals distinctive results not only to 5-HT1A receptor targeting but also between studies. Acute activation of the 5-HT2C receptor subtype has been shown to decrease cell proliferation resembling the effects of 5-HT1A antagonist or has no effect (Banasr et al., 2004, Klempin et al., 2010). Vice versa, acute blocking produces more BrdU-labeled cells lacking antagonistic action. We proposed that acute activation of one receptor subtype (5-HT1A) stimulates the production of cells that is lost after prolonged stimulation; yet, the increase in cell numbers is affected by another receptor subtype (5-HT2C) that stimulates both the number of proliferating late-stage precursor cells and net adult neurogenesis (Klempin et al., 2010). Neurosphere cultures also demonstrate the differential contribution of 5-HT1A and 5-HT2 receptors in both self-renewal of precursor cells, and promoting neuronal differentiation (Klempin et al., 2010). Nevertheless, results on receptor targeting seem to depend on the experimental design and the drugs used, in addition to possible interaction of glutamatergic and serotonergic signaling (McEwen et al., 2010).

Together with SERT 5-HT receptors are also targets for pharmacotherapy: In depression, SSRIs are thought to primarily inhibit serotonin reuptake into the presynaptic cell and to target 5-HT1A auto-receptors (Descarries and Riad, 2012); 5-HT2 receptors can be targets in obesity and following stroke as increased 5-HT2 receptor density seems to correlate with attenuation of symptoms of depressed mood that goes along with these diseases (Nigro et al., 2013). It has been further shown that in the absence of functional 5-HT2B receptors extracellular serotonin levels are reduced while treatment with selective 5-HT2B agonists induces an antidepressant-like response (Diaz et al., 2012). Studies also described the impact of the 5-HT2C receptor subtype that mimics the effect of antidepressant action by increasing precursor cell proliferation and survival (Soumier et al., 2009, Klempin et al., 2010). Recently, a study also proposed 5-HT4 receptor-mediated signaling in antidepressant action that reverses de-maturation of adult granule cells as this developed together with enhanced efficacy of serotonin-neuromodulation via 5-HT4 receptors (Kobayashi et al., 2010, Mendez-David et al., 2014). As we have seen, differential receptor targeting on sequential steps in the course of adult neurogenesis modulates proliferation and survival of hippocampal precursor cells (Figure 1B) (Brezun and Daszuta, 2000, Encinas et al., 2006, Klempin et al., 2010). However, the heterogeneity and varied localization of 5-HT receptor subtypes within the hippocampal niche has made it challenging to understand the site and mechanism of action of pharmacotherapy. Importantly, receptor activation is dynamic both temporally and spatially, and for future work on the receptors reviewed here and others it is likely to be necessary to develop a model of the signaling network created by the many receptor species.

*Animal models: addressing the controversy of serotonin's role in proliferation or survival of newly generated cells*

Serotonin functions in the CNS have been studied extensively by pharmacological approaches. Nowadays, new technologies give the advantage of generating animal models that allow selective manipulation of the serotonin system by gain- or loss-of-function studies in the brain. Genetic animal models have been developed that target serotonin receptor subtypes (5-HT1A), genes that are involved in serotonin synthesis (TPH2), vesicular packaging (VMAT2), or serotonin re-uptake (SERT). However, our knowledge regarding the role and effects on adult hippocampal neurogenesis in these models is still limited.

First approaches focused on the 5-HT1A KO mouse model due to its proposed predominant role in serotonin's influence on neurogenesis based on receptor targeting studies as described above. Despite the large number and variability of receptors in the DG, one receptor subtype seems to be sufficient to produce the effect on homeostasis

within the neurogenic niche (Santarelli et al., 2003). Although in this study, no differences in cell proliferation were observed in 5-HT1A KO animals compared to wild type, treatment with the selective 5-HT1A receptor agonist 8-OH DPAT confirmed its modulatory role, as no stimulation of proliferation in 5-HT1A KO mice was seen (Santarelli et al., 2003).

The advantage of other mouse models that are developmentally depleted in serotonin supply has been recently discovered. Elucidation of adult hippocampal neurogenesis revealed no measurable changes in baseline proliferation of precursor cells in mice that are almost devoid of brain serotonin ($Tph2^{-/-}$, $VMAT2^{SERT-Cre}$) or with a drastic reduction in serotonin levels (*Tph2KI, Pet1$^{-/-}$*) (Diaz et al., 2013, Klempin et al., 2013, Sachs et al., 2013). Considering the results reviewed above on pharmacological depletion of serotonin signaling that leads to a decrease in neurogenesis, it is surprising that sustained neurogenesis can occur in the absence of serotonin. This is most probably a result of developmental compensation evoked by its life-long depletion/reduction. The mechanism of compensation could have significant clinical relevance for restoring serotonin homeostasis in models of disease. For *Tph2$^{-/-}$* mice, although no change in overall proliferation was detected, there is a deficiency in the transition from type-1 stem cells to type-2 progenitors with an altered balance of precursor cell proliferation and cell death rate, accompanied by changes in the number of Sox2-expressing cells (Klempin et al., 2013). We suggested population changes on the cellular level in the absence of serotonin as potential compensatory mechanism that maintains the progenitor cell pool and thus homeostasis in *Tph2$^{-/-}$* mice. Whether compensation is also due to adaptations at the level of 5-HT receptors and their downstream pathways, or alterations in neurotrophic factor signaling such as an increase in hippocampal brain-derived neurotrophic factor (BDNF) expression (Migliarini et al., 2012, Sachs et al., 2013) induced by developmental deficiency in brain serotonin needs to be determined. In the model of congenital serotonin deficiency (*Tph2KI*), increased numbers of DCX-expressing cells have been found showing a shift towards late-stage progenitor cells (Sachs et al., 2013).

Surprisingly, too, evaluations of the survival and differentiation of newly generated neurons show a positive effect by increased cell numbers in models of decreased serotonergic transmission, e.g., *Tph2KI*, *Pet1$^{-/-}$*, $VMAT2^{SERT-Cre}$ (Diaz et al., 2013, Sachs et al., 2013) that is normalized by chronic treatment with 8-OH DPAT in *Pet1$^{-/-}$* mice (Diaz et al., 2013). Increased survival in these models that was also seen following chronic serotonin inhibition by PCPA in one study (Diaz et al., 2013) argues for a compensatory effect in these mice where loss of newborn neurons normally balances neurogenesis (Biebl et al., 2000). However, mouse models with life-long depletion in central serotonin exhibit effects that differ from data and knowledge gained by pharmacological manipulation studies. A thorough examination of altered modulation of adult neurogenesis in mice with constitutive

versus inducible serotonin deficiency is necessary to differentiate between acute and life-long effects of low serotonin availability.

Serotonin depletion in transgenic mouse models also results in altered behavior such as exaggerated aggressiveness that goes along with reduced anxiety (Mosienko et al., 2012) and increased impulsivity (Angoa-Perez et al., 2012). Increased escape-like reactions have been observed in the tail suspension test in $VMAT2^{SERT-Cre}$ (Narboux-Neme et al., 2011) but not $Tph2^{-/-}$ mice (Mosienko et al., 2012) revealing conflicting results. Nevertheless, brain serotonin-deficient mice do not exhibit the expected 'depression model' phenotype (reviewed in (Fernandez and Gaspar, 2012, Mosienko et al., 2014); that may reflect unchanged baseline neurogenesis, but also indicates an uncoupling of the behavioral mechanisms governing depression and serotonin levels in a unique homeostasis created over time. Compensatory mechanisms seem to occur in life-long serotonin depletion and the replacement of this neurotransmitter by expression or activity of other neurotransmitter systems has been debated (Beckman and Santos, 2013). In $Tph2^{-/-}$ mice, the hippocampus reveals a reduction in norepinephrine (NE) or dopamine (DA) levels (Gutknecht et al., 2012) that could partly mediate an effect on adult neurogenesis. However, data reviewed here show no changes in cell proliferation in serotonin-deficient mouse models at baseline arguing that reduced NE or DA concentrations in these animals do not affect homeostasis of neurogenesis.

Noticeable, serotonergic neurons and fibers persist in the adult brain of serotonin-depleted mice (Gutknecht et al., 2008, Alenina et al., 2009, Migliarini et al., 2012) raising the question of their functional role when serotonin is absent. The neuromodulator character of serotonin affects excitatory or inhibitory release mediated by glutamate or GABA (Schmitz et al., 1995, Li et al., 2000) with one study showing that serotonergic neurons itself can express the GABA-synthesizing enzyme glutamic acid decarboxylase (Fu et al., 2010). Furthermore, two-thirds of dorsal raphe Pet1 neurons exhibit vesicular glutamate transporter 3 that is required for glutamate release (Hioki et al., 2010, Liu et al., 2014). In a recent study, these neurons revealed rapid changes in neuronal activity during a reward test by releasing both serotonin and glutamate (Liu et al., 2014). When serotonin is absent, nerve fibers may retain a default phenotype, where co-transmitters continue to be regulated normally. Yet, data on the non-/existence of co-transmission of serotonin and neuropeptides such as somatostatin, galanin or substance P in the dorsal raphe are obscure and vary between species with a tendency to 'no evidence' as shown in a recent study (Fu et al., 2010).

Another genetically modified mouse model that is deficient in SERT ($5-HTT^{-/-}$) exhibits increased extracellular serotonin levels, and reveals increased cell proliferation in aged but not adult mice (Schmitt et al., 2007). Like-wise, an age-dependent increase in the anxiety-like behavior was observed associated with changes in spine density in the

prefrontal cortex and amygdala (Sakakibara et al., 2014). These results argue for another, undetermined chronic compensatory pathway induced by changes in serotonin levels. Although survival and differentiation of newborn neurons were not affected, increased numbers of proliferating cells have been observed in the CA3 region of mouse hippocampus (Karabeg et al., 2013), whereas adult rat deficient in SERT showed increased numbers of immature neurons expressing DCX in the DG (Schipper et al., 2011). SERT knockdown by siRNA in adulthood also contributes to antidepressant action (Ferres-Coy et al., 2013). However, data gained from this mouse model are few and further research is necessary to better define its phenotype.

*Neurogenesis/monoamine/neurotrophin hypothesis of antidepressant action*
Most studies on serotonin function in the brain focus on its correlation with neurogenesis and clinical disorders. The "neurogenesis or monoamine hypothesis" of depression is supported by the observation that most commonly used antidepressant drugs target the serotonin system and facilitate adult hippocampal neurogenesis (reviewed in (Kempermann and Kronenberg, 2003, Warner-Schmidt and Duman, 2006, Czeh and Lucassen, 2007, Sahay and Hen, 2007). In turn, a decline in neurogenesis accompanied by low levels of critical neurogenic modulators is associated with mood disorders. Depression has become a common disease in industrialized countries affecting a growing number of its population. Major depression can also develop following dementia or stroke and goes along with anxiety, alcohol dependence, and other psychiatric disorders. In the clinics modern antidepressants are used efficiently with the immediate action to alter the synaptic availability of the monoamine serotonin. However, today's serotonin pharmacotherapy is based on treatment outcome that lacks a clear understanding of the underlying therapeutic mechanisms but also the pathways that produce counter-indications, and research into the mechanisms of antidepressant action has been challenging.

As described above SSRIs are thought to target the serotonin system by inhibiting SERT function and rely on possible long-term adaptations of 5-HT pre-, and post-synaptic receptor subtypes. 5-HT1A, 5-HT2B, 2C and 5-HT4 receptors are soaring candidates for contribution to the antidepressant response, as they regulate normal development of dendritic spine density and synapse formation of pyramidal and granule cells in the DG, elicit long-term plastic changes that decrease anxiety-like behavior, and mediate normal maturation of late-stage progenitor cells (Yan et al., 1997, Santarelli et al., 2003, Banasr et al., 2004, Klempin et al., 2010, Kobayashi et al., 2010, Diaz et al., 2012, Mendez-David et al., 2014). In human studies, increased angiogenesis correlates with increased numbers of neural precursor cells in the DG following SSRI or tricyclic antidepressant (TCA) treatment (Boldrini et al., 2012).

Prolonged treatment with the commonly used drug fluoxetine (Flx) has been shown to increase survival of newly generated neurons in the DG, whereas short-term treatment had no effect (Malberg et al., 2000). Depending on the design, BrdU injections given before and after chronic treatment with Flx show increased precursor cell proliferation and number of newborn neurons as result of the antidepressant treatment (Malberg et al., 2000, Santarelli et al., 2003, Encinas et al., 2006, Klempin et al., 2010), whereas survival of BrdU-positive cells is only detected 28 days post-injection of BrdU that followed long-term treatment with Flx (Malberg et al., 2000). Chronic treatment also accelerates synaptogenesis and increased LTP and behavior in the hippocampal neurogenic niche (Wang et al., 2008). It might also affect overall relative composition of the precursor cell pool that led to symmetric divisions of an early progenitor cell class (Encinas et al., 2006). The delay in neurogenic response is a potential mechanism to explain the latency in the clinical effect of antidepressants (Klempin et al., 2010), and we have indicated this to be a balanced interplay of 5-HT1A and 5-HT2C receptor subtypes (as described in the paragraph above). Other recent studies reveal Flx's additional effect on DA receptors, and levels of neurotrophic signaling (Kobayashi et al., 2012, Lesemann et al., 2012). In accordance to the delayed effects of antidepressant action, a crucial involvement of cyclic AMP regulatory element-binding protein (CREB) (Palmer et al., 1997, Chen et al., 2001) and non-monoamine-based antidepressants have been suggested (Berton and Nestler, 2006).

Recently, the role of neurotrophins in the pathology of depression has been discussed with BDNF as critical modulator. Both signaling systems, serotonin and BDNF, are involved in the regulation of neural circuitries and antidepressant action, and also co-regulate each other (Mattson et al., 2004, Ferres-Coy et al., 2013). A decline in BDNF signaling goes along with neurodegeneration and behavioral changes related to chronic stress, whereas re-establishing BDNF levels may be the cause of therapeutic responses to antidepressants (Duman et al., 1997). Acute treatment with the 5-HT2A/C agonist DOI has been shown to decrease BDNF mRNA levels in the granule cell layer of the DG, while chronic administration results in desensitization of BDNF signaling (Vaidya et al., 1997). A novel antidepressant drug, agomelatine increases the survival of cells via melatonergic receptor agonistic, and 5-HT2C receptor antagonistic action that is further accompanied by increased BNDF signaling (Soumier et al., 2009). Furthermore, increased serum BDNF levels have been found during SSRI treatment in patients (Molendijk et al., 2011). BDNF signaling may also contribute to the latency effect since acute injection of BDNF into the hippocampus seems to counteract early effects of the SSRI by increasing SERT function (Benmansour et al., 2008). The delay is reduced by acute knockdown of SERT that leads to enhanced serotonin release accompanied by increased neurotrophic signaling and neurogenesis (Ferres-Coy et al., 2013). Further, therapeutic activity of antidepressant

drugs might result from an increase in BDNF synthesis in astrocytes (Quesseveur et al., 2013).

However, the effects of BDNF on adult hippocampal neurogenesis remain controversial (Deltheil et al., 2008). We have recently shown, that brain serotonin deficiency has no effect on baseline neurogenesis that may be compensated by altered neurotrophic/BDNF signaling. We have proposed BDNF as candidate for permanent compensation in serotonin deficient mice and recent data already reveal enhanced BDNF mRNA levels in *Tph2*$^{-/-}$ and *Tph2KI* mice (Migliarini et al., 2012, Sachs et al., 2013), although accompanied by increased serotonin fiber density in the hilus. Nevertheless, whether BDNF signaling is related to the clinical features of depression and whether distinct antidepressants directly affect BDNF equally to serotonin remains unknown. Yet, not all patients respond to the available drugs, and recent studies challenge the 'monoamine/neurotrophin hypothesis' by suggesting a more direct change in cellular plasticity such as spine density, the ceramide system or AMPA receptors as target for antidepressants (Gulbins et al., 2013). Investigating hippocampal tissue of depressed patients thus translating animal studies into human reveals that the number of putative stem cells is reduced but neither proliferation nor net neurogenesis is affected. Vice versa, antidepressant treatment with the classical drugs does not result in increased or normalized cell proliferation suggesting a neurogenesis-independent mechanism (David et al., 2009, Lucassen et al., 2010). A new therapeutic approach is the treatment with tianeptine, a modified TCA that functions independently from the serotonin system. Chronic treatment overcomes the stress-induced reduction of precursor cell proliferation in tree shrews while pharmacological properties suggest its action through the glutamatergic system with alterations in BDNF release (Czeh et al., 2001, McEwen et al., 2010).

*Serotonin function in the adult hippocampus*

Cell genesis in the adult hippocampus plays a key role in learning and memory, is modulated by serotonin, and involved in antidepressant action. Increased neurogenesis leads to more efficiency in pattern separation (Sahay et al., 2011). The advantage of continues generation of neurons lies in the immediate or long-term adaption to neurogenic stimuli (Kempermann et al., 1997, van Praag et al., 1999), injury, or pathology (Parent et al., 1997, Duman et al., 2001). This also includes the response to exogenous stimuli like physical exercise that could lead to the development of alternative approaches in treatment of neurodegenerative disorders. Running has long been known to provoke increased cell proliferation in the hippocampal niche (van Praag et al., 1999, Kronenberg et al., 2003). Taking advantage of the genetic loss-of-function model *Tph2*$^{-/-}$, we measured cell proliferation and differentiation in the adult DG following short-term voluntary wheel running (Klempin et al., 2013), and we established that serotonin is necessary for a fast

neurogenic response of the niche to changes in physical activity. This new evidence for a mechanism of serotonin in brain function elucidates one of its broad modes of action in the adult. This knowledge may lead to develop specific pathways that mediate exercise-dependent cell genesis in the hippocampus that will also have therapeutic relevance. Other studies support these findings showing that exercise-plus-antidepressant challenges with commonly used drugs in depression, e.g. citalopram or the NE-selective antidepressant reboxetine significantly increase BDNF levels in hippocampal regions (Russo-Neustadt et al., 1999, Russo-Neustadt et al., 2004). Yet, consequence of chronic loss of serotonin pathways in hippocampal function seems not tied to homeostatic mechanisms such as compensation by other neurotransmitter systems but more likely to an organism response to environmental changes (Klempin et al., 2013). The long-term goal is to identify the pathways of serotonin action to facilitate the design of alternative approaches for the treatment of depression or age-related decline in learning and memory.

**Concluding remarks**

In this chapter we have considered the critical role of serotonin and its receptors in the regulation of adult hippocampal neurogenesis (Table 1). Given the potentially central role of neurogenesis in cognition and emotional state, it is necessary that research continues to open our understanding of the molecular and pharmacological network by which serotonin regulates the hippocampal niche. Serotonin's importance in the modulation of adult neurogenesis was first proposed 15 years ago. Since then a growing number of pharmacological experiments confirmed its positive impact on cell proliferation and survival of newborn neurons. However, here we have identified apparent contradictions and divergent hypotheses in the literature, particularly in chronic models of serotonin manipulation. The vast number of receptor subtypes and the complex distribution of the serotonin system in the brain is likely a major reason for discordance that will be resolved in future research. Analyses of the genetically modified mouse models that are depleted in brain serotonin from early development throughout adulthood exhibit unexpected phenotypes in comparison to predictions derived from the interpretation of pharmacological studies; in particular sustained baseline levels. The effects observed in these models support the important role of serotonin in adult neurogenesis, but cannot discriminate between phenotypes induced by serotonin *per se* and compensatory processes provoked by its life-long depletion. In turn, pharmacological studies show that acute and chronic modulations of serotonin in the adult lead to significant neurogenic modification but do not reveal the target or the compensatory changes in targets over time.

We conclude that future work should be focusing on the sources of serotonin and the physiologic mechanisms whereby it is released onto hippocampal targets. While pharmacological and genetic studies are beginning to define the location and role of

specific 5-HT receptor subtypes in hippocampal cell genesis, data regarding the downstream regulatory networks is notably slim. A better understanding of paracrine and autocrine factors that are serotonin-dependent is necessary for the future development of a model of serotonergic/neurogenic mechanisms. Newly generated mouse models that can be targeted by optogenetic activation, or silencing of serotonergic fibers that project into the hippocampus are important next steps to elucidate serotonin signaling in the neurogenic niche. At last, a working model that accounts for the temporal and spatial role of serotonin and its receptor targets within the hippocampal niche will likely lead to better therapeutic designs for the treatment of cognitive, memory and mood disorders.

**Table 1 Summary of recent findings on serotonin influence and modulation of adult hippocampal neurogenesis.**

| Pharmacological serotonin depletion studies | | |
|---|---|---|
| Brezun & Daszuta 2000 | 5,7-DHT treatment | Raphe lesions lead to decreased 5-HT fiber density in the DG associated with decreased number of neurons in GCL and SVZ |
| Brezun & Daszuta 2000 | Raphe Transplantation | Raphe grafts restore serotonergic control on precursor cell proliferation and reverse lesion-induced decrease |
| Huang & Herbert 2005 | PCPA treatment 5,7-DHT treatment | No effect on cell proliferation either 14 days (PCPA) or 3 weeks (5,7-DHT) after |
| Jha et al., 2006 | PCPA treatment 5,7-DHT treatment | Decreases precursor cell proliferation and survival Selectively decreases hippocampal 5-HT levels |
| Diaz et al., 2013 | PCPA treatment | Increases survival or newly generated neurons |
| Pharmacological receptor targeting studies | | |
| Yan et al., 1997 | 5-HT1A | Mediates the effect of serotonin depletion on decreased spine density of granule cells |
| Radley & Jacobs 2002 | 5-HT1A postsynaptic | Ant/agonist treatment affects number of BrdU-positive cells / modulates anxiety-related behavior |
| Banasr et al., 2004 | 5-HT1A, 5-HT1B, and 5-HT2C | Ant/agonist treatment reveals differential effects on SVZ and SGL newborn cells |
| Huang & Herbert 2005 | 5-HT1A postsynaptic | Agonist treatment following PCPA or 5,7-DHT modulates cell proliferation by a direct postsynaptic effect |
| Klempin et al., 2010 | 5-HT1A, 5-HT2 | Ant/agonist treatment differentially affects neurogenesis |
| Mogha et al., 2012 | 5-HT1A | 5-HT1A receptor signaling is important for normal synaptogenesis in the neonatal hippocampus |
| Mendez-David et al., 2014 | 5-HT4 | Facilitates maturation of newborn neurons in the DG |
| Mouse models with genetically modified serotonin system | | |
| Santarelli et al., 2003 | 5-HT1A KO mouse | 5-HT1A activation stimulates neurogenesis in the DG |
| Schmitt et al., 2007 | $SERT^{-/-}$ ($5\text{-}HTT^{-/-}$) mouse | Increased synaptic 5-HT levels influence proliferation in aged but not young adult and adult mice |
| Schipper et al., 2011 | $SERT^{-/-}$ rat | Increased numbers of DCX-positive cells in the DG |
| Migliarini et al., 2012 | Tph2::eGFP | Increased serotonergic innervation in nucleus accumbens and hippocampus |
| Diaz et al., 2013 | $Pet1^{-/-},$ $VMAT2^{SERT\text{-}Cre}$ | Increased survival or newly generated neurons at baseline |
| Klempin et al., 2013 | $Tph2^{-/-}$ mouse | Normal baseline proliferation in serotonin deficient mice |
| Sachs et al., 2013 | Tph2KI mouse | Increased survival or newly generated neurons at baseline with increased numbers of DCX-positive cells |
| Antidepressant action and neurogenesis | | |

| Malberg et al., 2000 | Flx | First shown increase in neurogenesis after prolonged treatment with Flx |
|---|---|---|
| Chen et al., 2001 | cAMP | cAMP signal transduction cascade contributes to increased neurogenesis as antidepressant response |
| Czeh et al., 2001 | Tianeptine | Treatment reduces proliferation of precursor cells in the DG |
| Santarelli et al., 2003 | 5-HT1A KO Flx | 5-HT1A receptors are required for Flx-induced hippocampal neurogenesis |
| Encinas et al., 2006 | Flx | Flx acts solely on type-2a progenitors by increasing the rate of symmetric cell divisions |
| Wang et al., 2008 | Flx | Chronic treatment accelerates maturation and synaptogenesis of immature granule cells |
| Benmansour et al., 2008 | SSRI | Acute BDNF injection into the hippocampus increases SERT function |
| Soumier et al., 2009 | 5-HT2C | Increases proliferation and mediates the antidepressant effect of agomelatine |
| Klempin et al., 2010 | 5-HT1A, 5-HT2C | Latency of Flx action due to additive effects of 5HT1A, 2C receptors |
| Kobayashi et al., 2010 | 5-HT4 KO Flx | Flx treatment reverses neuronal maturation (Calbindin expression) with up-regulated 5-HT4 signaling |
| Diaz et al., 2012 | 5-HT2B | Pharmacological receptor stimulation mimics SSRI-like response |
| Boldrini et al., 2012 | SSRI, TCA | Stimulation of angiogenesis and neurogenesis |
| Ferres-Coy et al., 2013 | RNAi – SERT | Acute SERT silencing increases 5-HT release and neurogenesis and decreases latency in antidepressant action |
| Vaidya, Marek et al. 1997 | BDNF | Decreased BDNF mRNA levels in the DG after 5-HT2A and 2C chronic agonist treatment |
| Deltheil et al., 2008 | BDNF | BDNF potentiates the effect of SSRI treatment (*in vivo* intracerebral microdialysis) |
| Quesseveur et al., 2013 | BDNF | SSRI treatment increases BDNF levels released from astrocytes that promote neurogenesis |
| **Functional role of serotonin in adult neurogenesis** | | |
| Russo-Neustadt et al., 2004 | SSRI, BDNF, exercise | Exercise-plus-antidepressant challenge led to increased BDNF levels in the hippocampus |
| Klempin et al., 2013 | *Tph2*$^{-/-}$ mouse | No running-induced effect on proliferation when serotonin is absent |

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## Glossary:

5-HT: 5-hydroxytryptamine, serotonin

5,7-DHT: 5,7-dihydroxytryptamine, a neurotoxin that selectively is taken up by SERT and causes neuronal damage upon MAO-A-mediated oxidation.

8-OH DPAT: 8-hydroxy-2-(dipropylamino)tetralin hydrobromide, standard selective 5-HT1A agonist

BDNF: brain-derived neurotrophic factor

BrdU: bromodeoxyuridine, a thymidine analog that gets incorporated into the replicating DNA (during the S-phase of the cell-cycle) and is commonly used for the detection of proliferating cells.

cAMP: cyclic AMP or 3'-5'-cyclic adenosine monophosphate

DCX: doublecortin, microtubule associated protein and transient marker of immature neurons in the DG

DG: dentate gyrus

Flx: fluoxetine, SSRI that is widely used as antidepressant (trade name Prozac)

LTP: long-term potentiation

PCPA: para-chlorophenylalanine, TPH inhibitor

Pet1: ETS (E-twenty six) transcription factor, expressed predominantly in serotonergic neurons

SERT (Slc6a4, 5-HTT): serotonin transporter, expressed predominantly in serotonergic neurons

SGZ: subgranular zone

SSRI: selective serotonin reuptake inhibitor, treatment increases extracellular serotonin availability immediately

Sox2: SRY-related HMG-box gene 2, transcription factor that drives the cell fate of precursor cells toward an astrocyte lineage, in the DG it is mainly expressed in stem and type-2a progenitor cells

SVZ: subventricular zone

TCA: tricyclic antidepressant

TPH2: tryptophan hydroxylase 2, rate-limiting enzyme of serotonin synthesis in the brain, expression exclusively in serotonergic neurons

VMAT2: vesicular monoamine transporter 2, responsible for packing serotonin into synaptic vesicles, expression in serotonergic, but also dopaminergic and noradrenergic neurons

## Mouse models:

*VMAT2$^{SERT-CRE}$*: mouse with conditional deletion of VMAT2 in serotonergic neurons that had been crossed by VMAT2$^{flox/flox}$ and SERT-Cre mouse lines (expressing Cre recombinase under the control of the SERT promoter); uniform reduction of serotonin in all brain regions (more than 95%)
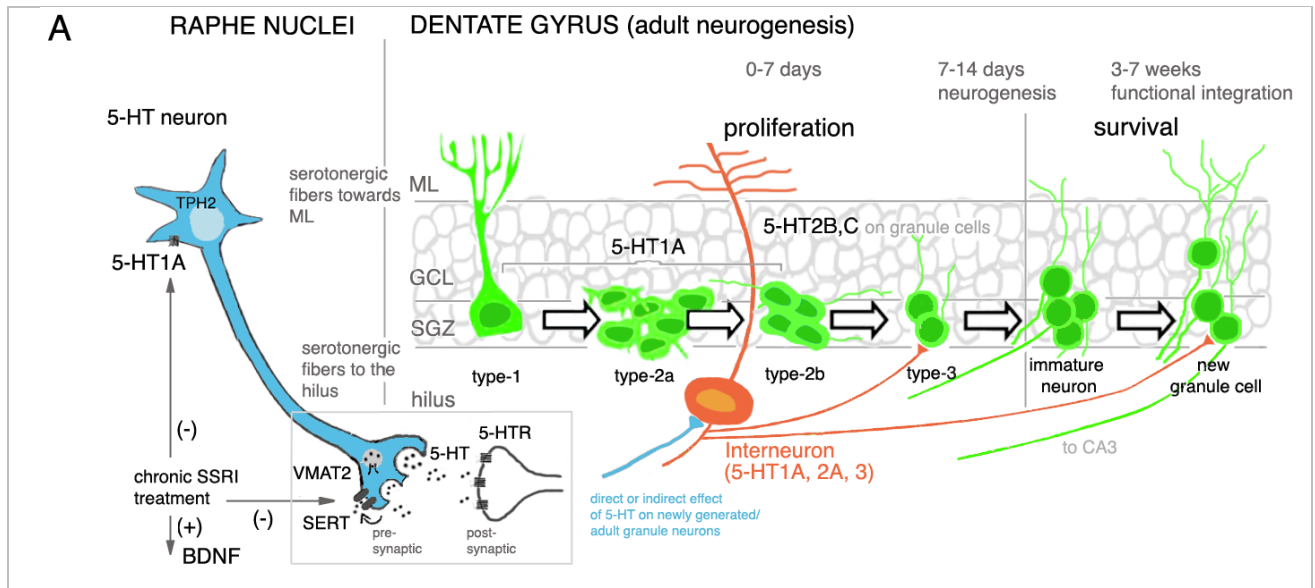
*Tph2$^{-/-}$: Tph2*-deficient mouse with constitutive ablation of TPH2 enzyme that leads to serotonin depletion in the brain (more than 95% reduction)

*Pet1$^{-/-}$*: hypo-serotonergic mouse model in which the majority (80%) of serotonergic neurons in the raphe fail to differentiate (85% serotonin reduction)

*Tph2KI*: *Tph2* R439H knock in, hypo-serotonergic mouse bearing a single-nucleotide mutation, equivalent to a rare human variant (R441H) identified in depressed patients (80% serotonin reduction).

*5-HTT$^{-/-}$*: *Sert*-deficient mouse with 60-80% reduction in brain serotonin level, but about 10-fold increase in extracellular serotonin concentrations

*Tph2*::eGFP: *Tph2*-deficient mouse model that carries an additional eGFP cDNA knocked in into the *Tph2* locus and allows the visualization of serotonergic fibers in *Tph2$^{+/-}$* and *Tph2$^{-/-}$* mice.

**A**    RAPHE NUCLEI     DENTATE GYRUS (adult neurogenesis)

*Panel A labels:* 5-HT neuron; TPH2; 5-HT1A; serotonergic fibers towards ML; serotonergic fibers to the hilus; chronic SSRI treatment; (−); (+); (−); BDNF; VMAT2; SERT; 5-HT; pre-synaptic; post-synaptic; 5-HTR; ML; GCL; SGZ; hilus; 0-7 days; 7-14 days neurogenesis; 3-7 weeks functional integration; proliferation; survival; 5-HT1A; 5-HT2B,C on granule cells; type-1; type-2a; type-2b; type-3; immature neuron; new granule cell; Interneuron (5-HT1A, 2A, 3); direct or indirect effect of 5-HT on newly generated/adult granule neurons; to CA3

**B**

| Pharmacological manipulation | Drug effect | type-1 | type-2a/2b | type-3 | net neurogenesis | BDNF |
|---|---|---|---|---|---|---|
| 5,7-DHT | Lesion of 5-HT neurons | n/a | – | – / 0 | – / 0 | |
| PCPA | Inhibition of 5-HT synthesis | – | – | – | – / + | |
| 5-HT1A (postsynaptic) | Stimulation | n/a | ++ | + | + | |
| | Blockage | – | – | – | – | |
| 5-HT1B | Stimulation | n/a | + | n/a | n/a | |
| 5-HT2A | Stimulation | n/a | – | n/a | – | ↓ |
| | Blockage | | + | 0 | 0 | |
| 5-HT2B | Stimulation | Induces antidepressant-like response | | | | |
| | Blockage | Decreases 5-HT levels | | | | |
| 5-HT2C | Stimulation | – | – / 0 | + | ++ | ↓ |
| | Blockage | n/a | + | 0 | 0 | ↑ |
| 5-HT4 | Stimulation | n/a | n/a | + | + | |
| SSRI-Flx[1] | Increases extra-cellular 5-HT levels | No effect | ++ | + | ++ | ↑ |
| *Animal models* | *Phenotype* | | | | | |
| 5-HT1A KO | | 0 | 0 | n/a | n/a | |
| Tph2^{-/-} | No serotonin | 0 | Increased Sox2 | 0 | 0 | ↑ |
| VMAT2[SERT-Cre] | Reduced 5-HT levels | 0 | 0 | 0 | ++ | |
| Tph2KI | Reduced 5-HT levels | n/a | 0 | + | ++ | ↑ |
| Pet1^{-/-} | Reduced 5-HT levels | n/a | 0 | n/a | ++ | |
| SERT^{-/-} | Increases extra-cellular 5-HT levels | n/a | + | + | n/a | |

[1] refer for summary to Klempin et al. 2010

**Figure 1**

**Illustration of serotonin's (5-HT) mode of action in the course of adult hippocampal neurogenesis.**

**A.** Serotonergic neurons are located in the brainstem raphe nuclei where 5-HT is synthesized by TPH2, packaged into vesicles and transported by VMAT2, while its re-uptake into the presynaptic neurons is ensued by its selective transporter (SERT) (the embedded square marks a synapse). SERT and presynaptic 5-HT1A autoreceptors on serotonergic neurons are targets for SSRIs, which may also modulate BDNF levels. Dense tracts of serotonergic fibers terminate in the hippocampus

with one projecting into the molecular layer (ML) and another to the hilus, where it synapses on principle neurons and interneurons (in orange). Hilar interneurons in the dentate gyrus (DG) in turn contact immature and newly generated granule cells suggesting an indirect effect of serotonin action. In the DG, type-1, type-2a/b, type-3, immature and mature neurons mark the six developmental steps within adult neurogenesis. Type-2a cells are highly proliferative. The variety of 5-HT receptors in the DG is differentially expressed with 5-HT1A receptor immunolabeling found on type-1 to type-2b cells in the subgranular zone (SGZ), and hilar interneurons. 5-HT2B and 2C receptors mostly mark adult granule cells whereas 5-HT2A receptor labeling reveals a dense staining in the hilus; 5-HT3 receptor expression was seen on hilar interneurons. BDNF, brain-derived neurotrophic factor; GCL, granule cell layer; SSRI, selective serotonin re-uptake inhibitor; TPH2, tryptophan hydroxylase 2; VMAT2, vesicular monoamine transporter 2. **B.** The table summarizes the effect of pharmacological targeting and genetic manipulation of the serotonin system on the generation of newborn neurons. Animal models developmentally manipulated in brain serotonin do not show a drastic change in adult neurogenesis but reveal long-term effects on the survival of newborn neurons and neurotrophin signaling (BDNF). *Characters:* +/++ or – refer to increased cell numbers/positive effects or decreased cell numbers/negative effects; 0 no change; n/a not analyzed; ↑↓ increased or decreased BDNF levels in the hippocampus