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# Habilitationsschrift

# Signaling Pathways Linking Behavior to Neurogenesis in Healthy Brain and Disease

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for my phd-students

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# Contents

# Abbreviations

| | |
|---|---|
| Alzheimer's disease | AD |
| Angiotensin | Ang |
| Angiotensin converting enzyme 2 | ACE2 |
| Bradykinin receptors 1 and 2 | B1R, B2R |
| Brain-derived neurotrophic factor | BDNF |
| Bromodeoxyuridine | BrdU |
| Central nervous system | CNS |
| Dentate gyrus | DG |
| Electroconvulsive (seizure) therapy | ECS, ECT |
| Induced pluripotent stem cell | iPSC |
| Neural stem cell | NSC |
| Renin-angiotensin system | RAS |
| Selective serotonin reuptake enhancer | SSRE |
| Selective serotonin reuptake inhibitor | SSRI |
| Serotonin | (5-hydroxytryptamine, 5-HT) |
| Serotonin transporter | SERT (5-HTT, SLC6A4) |
| SRY-related HMG-box gene 2 | Sox2 |
| Subgranular zone | SGZ |
| Tryptophan | Trp |
| Tryptophan hydroxylase 2 | TPH2 |

# 1. Introduction

The adult brain is highly plastic. Its structure is able to adapt to environmental challenges and novel experiences by rewiring upon learning, and responds to trauma or injury. The brain's *'neuroplasticity'* comprises alterations in synaptic structure, strengthening or weakening synaptic connections, but also the generation of new neurons and creation of new neural connections. Specifically, the discovery that new neurons are continuously generated throughout life has stirred hope for new therapeutic strategies to improve cognitive function and to treat neurodegenerative disorders.

## 1.1. From Stem Cells to Signaling Pathways Regulating Adult Neurogenesis

The dentate gyrus (DG) of the hippocampus is one of the two prominent regions in the mammalian brain where '*adult neurogenesis'* persists throughout life (Altman and Das, 1965; Kaplan and Hinds, 1977). As part of the limbic system, the hippocampus plays a central role in learning, especially in the encoding and retrieval of episodic and spatial memories (Buzsaki and Moser, 2013). While synaptic plasticity and dendrite complexity, GABA and cholinergic tone drive the neural circuit of memory formation (Buzsaki, 1989; Hasselmo, 1999), continued neuron generation might also contribute to learning, and adding new neurons might lead to optimizing a running system upon environmental need (Kempermann, 2002). The neurogenic niche of the DG, the subgranular zone (SGZ), accommodates neural stem cell (NSC) populations (type-1 cells) that give rise to proliferating amplified progenitor cells (type-2a/b, type-3) that become immature and mature neurons (Kempermann *et al*, 2004) in a time-specific manner (Plumpe *et al*, 2006; van Praag *et al*, 2002). The progression is regulated dynamically within a defined microenvironment that is primed to support a neuronal fate. Surrounded by vasculature and in

close proximity to glial cells, neural stem/progenitor cells retain fate plasticity and respond to a variety of local cues and extrinsic stimuli that promote neurogenesis.

Among the neurogenic niche's intrinsic components are cell-autonomous molecules and extracellular signaling proteins, growth and transcription factors that differ in their effects on stem cell maintenance, cell proliferation and differentiation, cell death and survival (reviewed in Goncalves *et al*, 2016). To regulate the distinct stages of adult neurogenesis, coordinated and cell type-specific gene transcription is required. Sox2 (SRY-related HMG-box gene 2) is a prominent regulator that controls multipotency and fate choice of type-1 and type-2 precursor cells (Steiner *et al*, 2006). In concert with lineage instruction factors, pathways driven by serotonin or neurotrophic signaling, e.g. brain-derived neurotrophic factor (BDNF) signaling, are key regulators to maintain neurogenesis. While serotonin modulates both proliferation and survival of newly generated cells (reviewed in Alenina and Klempin, 2015), BDNF is crucially involved in neuronal maturation and synaptic plasticity (Ferres-Coy *et al*, 2013; Mattson *et al*, 2004). Alongside local cues, novel experiences influence stem/progenitor cell behavior in rodents, and activity-dependent changes in neuroplasticity occur; including increased precursor cell proliferation upon physical exercise (Kronenberg *et al*, 2003; van Praag *et al*, 1999) and cell survival upon exposure to an enriched environment (Kempermann and Gage, 1999). Thereby, the various growth and neurotrophic factors participate in the experience-dependent effects on neurogenesis (Woost *et al*, 2018).

Neuroplasticity of the adult brain can also be negatively regulated; inducing structural changes that can be studied in the diseased brain. Impaired neurogenesis has been observed in stress-related events and is associated with age-related cognitive decline, neurodegenerative and psychiatric disorders in humans (e.g. Alzheimer's disease (AD), temporal lobe epilepsy, major depression), and obesity. Whether neuron loss is causative or associative with the pathologies still needs to be shown. Nevertheless, many of the intrinsic factors of the neurogenic niche also appear to be altered in disease. In the case of major depression, manipulations modulating extracellular serotonin levels lead to clinical improvement that is linked to a delayed increase in adult neurogenesis as shown in rodents (Malberg *et al*, 2000; Santarelli *et al*, 2003).

Furthermore, low serum BDNF levels have been observed in depressed patients that can be replenished by antidepressant treatment (Molendijk *et al*, 2011). While neuron replacement is the desired strategy to repair the diseased brain, targeting of signaling factors that support neuronal development is a potential therapeutic alternative.

Identifying serotonin's contribution to brain plasticity in health and disease has been a unifying component of my research career. Dysregulation of serotonin signaling is associated with neurogenic decline, age-related memory loss and mood disorders, but also impacts homeostatic systems that control satiety, energy metabolism and stress responses (reviewed in Donovan and Tecott, 2013). Serotonin is the most widespread monoamine of the central nervous system (CNS) with fiber pathways projecting into numerous brain areas and spinal cord. Target areas in the DG express various 5-HT receptors that control the response from efferent activity (Brezun and Daszuta, 2000; Klempin *et al*, 2010). Serotonin mediates a variety of functions but is particularly known for conveying a sense of contentment and happiness. Serotonin-based antidepressants are used with the immediate action to alter the synaptic availability of the monoamine, targeting serotonin transporter and 5-HT (auto-)receptors (reviewed in Descarries and Riad, 2012). '*State of the art*' therapy consists of selective serotonin reuptake inhibitors (SSRIs) or, somewhat paradoxically, also SSR-enhancers (SSREs). Although SSRI-mode of action is an acute facilitation of serotonin transmission, only chronic treatment leads to clinical improvement accompanied by increased cell proliferation and generation of new neurons in the DG (Encinas *et al*, 2006; Klempin *et al*, 2010; Malberg *et al*, 2000). However, a fairly large number of patients do not respond to treatment. Lack of understanding a clear mechanism of SSRI function significantly limits our ability to effectively manage depression.

Several approaches developed in animal models, e.g. acute depletion, or 5-HT receptor targeting (Brezun and Daszuta, 2000; Diaz *et al*, 2013; Klempin *et al*, 2010) have already been used in the clinics and *vice versa*. Recent genetically modified models that differ in their availability of serotonin, constitute new powerful tools to study neuroplasticity and drug development (Alenina and Klempin, 2015). Tryptophan hydroxylase 2 deficient (*Tph2*$^{-/-}$) mice

are selectively depleted of brain serotonin (Alenina *et al*, 2009). *Tph2*$^{-/-}$ mice exhibit transient early postnatal growth retardation, and modest impairment of sleep and respiration. In adulthood, *Tph2*$^{-/-}$ mice lack maternal care and reveal a less anxious but highly aggressive phenotype (Mosienko *et al*, 2012). Increased aggression and a despair-like state are significant of the clinical presentation of depressed patients with reduced serotonin function (i.e., low 5-hydroxyindoleacetic acid [5-HIAA] levels in cerebrospinal fluid (Mann and Malone, 1997; Placidi *et al*, 2001). Other genetically modified models comprise of overall reduced, or increased extracellular levels of serotonin, e.g. mice lacking serotonin transporter. To define mechanisms of pharmacotherapy manipulating serotonin remains in research focus since clinical improvement can still be accomplished by treatment with SSRI/SSRE in some patients. However, to discover the regulators and interactive pathways by which serotonin mediates brain function is of equal importance.

Insight comes from studying the contribution of various factors. Previous research has focused on serotonin alone. Current theories of the '*neurobiology of depression*' center on structural changes and plasticity dynamics, e.g. impaired adult neurogenesis, dysregulation of the hypothalamic pituitary adrenal axis, and altered BDNF signaling. While the biological systems are partly intertwined, they might rapidly become uncoupled in disease. BDNF is important for the neuronal development. In response to stress, BDNF levels in the hippocampus decrease which in turn might negatively regulate neuroplasticity (reviewed in Duman and Monteggia, 2006). Antidepressants targeting the serotonin system but also physical exercise significantly upregulate BDNF signaling (Adlard *et al*, 2005; Nibuya *et al*, 1996)–beneficially affecting mood. A few recent studies challenge the monoamine/neurotrophin hypothesis of depression by suggesting a direct change in spine density or the ceramide system as antidepressant targets (Gulbins *et al*, 2013; Panayotis *et al*, 2018). Other candidate molecules that might exhibit new drug targets include circulatory factors and signals released by skeletal muscles; e.g. vascular endothelial growth factor, the peptide hormone angiotensin (Ang) II and Cathepsin B, shown to modulate cell proliferation (Fabel *et al*, 2003; Moon *et al*, 2016; Mukuda *et al*, 2014), or vasoactive kinins which can also act as neuropeptides (reviewed in Negraes *et al*, 2015). Excitingly, most of these factors, central and peripheral, are involved in, or modulated

by physical exercise that in turn accelerates neuroplasticity of the hippocampus. The interplay between these various factors, between serotonin and BDNF, their effects on additional responses within the niche, e.g. neurogenesis, and in close proximity to the vasculature, needs to be determined and will be discussed in the following.

## 1.2. Objectives

My research has been inspired by the excitement surrounding adult neurogenesis and the hope that a few discrete NSC populations have the power to modify brain function on demand. My studies on stem cell biology particularly center on the role of serotonin–a crucial signal in the neurogenic niche microenvironment, also involved in antidepressant action–and focus on the intersection of changes in behavior such as physical activity and the regulation of neurogenesis in the DG. Elucidating basic neurobiological mechanisms of the niche, the effects mediated by physical activity, and targeting of central and peripheral signaling pathways (e.g. the novel candidate molecule BDNF, and circulatory factors), could lead to potent strategies to restore neurogenesis in people with low or inactive brain serotonin, such as in major depression.

By employing a combination of genetic tools and behavioral paradigms the following research provides insight into

i) principles of lineage progression of newly born cells in the hippocampus
ii) the effects of physical exercise on neurobiological mechanisms
iii) the interplay of serotonin and BDNF under physiological conditions, and how they might become uncoupled in disease
iv) targeting of novel signaling pathways that support neuronal development as potential therapeutic alternative.

# 2. Original research

## 2.1. Lineage-Instruction Factors of the Neurogenic Niche

The approach to drive fate *de novo* from stem and non-stem cells of the (diseased) brain independently of the use of embryonic or induced pluripotent stem cell (iPSC) replacement is of particular clinical significance. This potential therapeutic strategy of fate respecification could avoid immunogenicity and time grafting exogenous cells. The most attractive approach appears to be direct *in vivo* reprogramming of resident neural cells to induce a neuronal fate (Gascon *et al*, 2016; Klempin *et al*, 2017). Knowledge can be gained from studying neurogenic niches whose innately regenerative capacity is distinguished from non-neurogenic or glial regions of the adult brain. Set in a microenvironment of factors and signaling that dictate the rate and type of cells developed, NSCs retain fate plasticity and respond to stimuli that foster neurogenesis.

The transcription factors and lineage-related genes Olig2 and Pax6 participate in the progression from type-1 stem cells to mature neurons in the DG, and manipulating their activity can direct the outcome of neurogenesis.

The following abstract is from the original research article:

"**Klempin F**, Marr RA, Peterson DA (Modification of pax6 and olig2 expression in adult hippocampal neurogenesis selectively induces stem cell fate and alters both neuronal and glial populations. *Stem Cells* 30:500-509.2012; doi: https://doi.org/10.1002/stem.1005

> *"The generation of new neurons in the mammalian hippocampus continues throughout life, and lineage progression is regulated by transcription factors, local cues, and environmental influences. The ability to direct stem/progenitor cell fate in situ may have therapeutic potential. Using an in vivo retroviral delivery and lineage tracing approach, we compare the lineage-instruction factors, Pax6*

*and Olig2, and demonstrate that both participate in regulation of adult hippocampal neurogenesis in adult rats. We show that overexpression of the proneuronal factor Pax6 pushes neuronal precursor cells to early maturation and increases the frequency of neuronal phenotypes. However, Pax6 overexpression results in no net increase in neurogenesis at 3 weeks. Blocking of Olig2 function reduces and slows neuronal commitment and differentiation and decreases net neurogenesis. Altering expression of both factors also changes gliogenesis. Our results establish that Pax6 decreases the number of Neuron-Glia 2 progenitor cells and prevents oligodendrocyte lineage commitment, while repression of Olig2 results in an expanded astrocytic lineage. We conclude that selectively modifying transcriptional cues within hippocampal progenitor cells is sufficient to induce a cell fate switch, thus altering the neurogenesis–gliogenesis ratio. In addition, our data show the competence of multiple progenitor lineages to respond divergently to the same signal. Therefore, directing instructive cues to select phenotype and developmental stage could be critical to achieve precise outcomes in cell genesis. Further understanding the regulation of lineage progression in all progenitor populations within the target region will be important for developing therapeutic strategies to direct cell fate for brain repair."*

## 2.2. Serotonin Transduces Physical Activity into Precursor Cell Proliferation

In addition to cell-autonomous molecules and transcription factors, the neurogenic niche consists of signaling mediated by neurotransmitters, growth and neurotrophic factors. Serotonin crucially contributes to the hippocampus' capacity for adult neurogenesis, and has been intensely studied using pharmacological depletion or receptor targeting approaches (Brezun and Daszuta, 2000; Diaz *et al*, 2013; Klempin *et al*, 2010). Another pro-neurogenic stimulus is physical exercise, identified to strongly increase the number of proliferating cells in the DG (van Praag *et al*, 1999), filling up the niche's reservoir of new cells that can be recruited on demand into a neuronal fate (Fabel and Kempermann, 2008).

In a new and more direct approach, the unique genetic loss-of-function model, *Tph2*$^{-/-}$ mice (Alenina *et al*, 2009), was employed to study adult neurogenesis, and the effects of exercise on precursor cell proliferation in the absence of brain serotonin.

The following abstract is from the original research article:

"**Klempin F***, Beis D*, Mosienko V, Kempermann G, Bader M, Alenina N (Serotonin is required for exercise-induced adult hippocampal neurogenesis. *J Neurosci* 33:8270-8275.2013; doi: https://doi.org/10.1523/JNEUROSCI.5855-12.2013

*F.K. and D.B. contributed equally to this work; F.K. and D.B. designed and performed research, and analysed data; F.K. wrote the paper

Comment in: The importance of serotonin in exercise-induced adult neurogenesis: new evidence from *Tph2*$^{-/-}$ mice. [J Neurosci. 2013]

> *"Voluntary wheel running has long been known to induce precursor cell proliferation in adult hippocampal neurogenesis in rodents. However, mechanisms that couple activity with the promitotic effect are not yet fully understood. Using tryptophan hydroxylase (TPH) 2 deficient (Tph2-deficient) mice that lack brain serotonin, we explored the relationship between serotonin signaling and exercise-induced neurogenesis. Surprisingly, Tph2-deficient mice exhibit normal baseline hippocampal neurogenesis but impaired activity-*

*induced proliferation. Our data demonstrate that the proproliferative effect of running requires the release of central serotonin in young-adult and aged mice. Lack of brain serotonin further results in alterations at the stage of Sox2-positive precursor cells, suggesting physiological adaptations to changes in serotonin supply to maintain homeostasis in the neurogenic niche. We conclude that serotonin plays a direct and acute regulatory role in activity-dependent dependent hippocampal neurogenesis. The understanding of exercise-induced neurogenesis might offer preventive but also therapeutic opportunities in depression and age-related cognitive decline."*

## 2.3. The Interplay between Brain Serotonin and BDNF Signaling

The various signaling molecules of the neurogenic niche collaborate with, and may compensate for the lack of the other. Particularly exciting is the observation that BDNF may be a compensatory target for reinvigorating depression-associated loss of neurogenesis. BDNF is the most abundant neurotrophin in the DG, and crucially involved in synaptic plasticity and in the generation and survival of newborn neurons (Ferres-Coy *et al*, 2013; Mattson *et al*, 2004). Numerous basic studies have reported decreased BDNF levels in the hippocampus in response to diverse stress conditions (Duman and Monteggia, 2006). Furthermore, SSRI-induced increases in serum BDNF have been detected in rodents (Nibuya *et al*, 1996) and similarly in depressed patients (Molendijk *et al*, 2011). Conversely, changes in serotonin neurotransmission may be induced by BDNF (Benmansour *et al*, 2008).

The next two studies examine the interplay of serotonin and BDNF, in particular as it relates to the effects of additional pro-/anti-depressive stimuli, e.g. adult neurogenesis.

### 2.3.1. Lack of Brain Serotonin Is Compensated by Alterations in BDNF Level

BDNF signaling could be relevant to mechanisms of antidepressant treatment targeting the serotonin system. Using two powerful genetic animal models of altered central serotonin signaling, this study determines and compares protein levels of the neurotrophic factor in different brain areas by ELISA technique.

The following abstract is from the original research article:
"Kronenberg G, Mosienko V, Gertz K, Alenina N, Hellweg R, **Klempin F** (Increased brain-derived neurotrophic factor (BDNF) protein concentrations in mice lacking brain serotonin. *Eur Arch Psychiatry Clin Neurosci*. 2016 Apr;266(3):281-4; doi:
https://link.springer.com/article/10.1007/s00406-015-0611-3

> *"The interplay between BDNF signaling and the serotonergic system remains incompletely understood. Using a highly sensitive enzyme-linked immunosorbent assay, we studied BDNF concentrations in hippocampus and*

*cortex of two mouse models of altered serotonin signaling: tryptophan hydroxylase (Tph)2-deficient ($Tph2^{-/-}$) mice lacking brain serotonin and serotonin transporter (SERT)-deficient ($SERT^{-/-}$) mice lacking serotonin re-uptake. Surprisingly, hippocampal BDNF was significantly elevated in $Tph2^{-/-}$ mice, whereas no significant changes were observed in $SERT^{-/-}$ mice. Furthermore, BDNF levels were increased in the prefrontal cortex of $Tph2^{-/-}$ but not of $SERT^{-/-}$ mice. Our results emphasize the interaction between serotonin signaling and BDNF. Complete lack of brain serotonin induces BDNF expression."*

### 2.3.2. Serotonin Critically Contributes to the Effects of Electroconvulsive Therapy

As shown above, my research indicates that serotonin significantly forms the brain's response to physical exercise. In the absence of the neuromodulator, neurotrophic signaling is highly active. Serotonin is also a main player in antidepressant action. Electroconvulsive therapy is a very effective treatment for major depression–a serious and highly prevalent mental disorder. The following study examines whether serotonin is critically involved in brain responses to electroconvulsive seizure, namely the induction of adaptive responses such as increased adult neurogenesis and increased BDNF signaling.

The following abstract is from the original research article:

"Kronenberg G, Petermann M, Dormann C, Bader M, Gass P, Hellweg R, **Klempin F** (Brain serotonin critically contributes to the biological effects of electroconvulsive seizures. *Eur Arch Psychiatry Clin Neurosci.* 2018 Dec;268(8):861-864; doi: https://doi.org/10.1007/s00406-018-0924-0

> *"Compounds targeting serotonin (5-HT) are widely used as antidepressants. However, the role of 5-HT in mediating the effects of electroconvulsive seizure (ECS) therapy remains undefined. Using Tph2$^{-/-}$ mice depleted of brain 5-HT, we studied the effects of ECS on behavior and neurobiology. ECS significantly prolonged the start latency in the elevated O-Maze test, an effect that was abolished in Tph2$^{-/-}$ mice. Furthermore, in the absence of 5-HT, the ECS-induced increase in adult neurogenesis and in brain-derived neurotrophic factor signaling in the hippocampus were significantly reduced. Our results indicate that brain 5-HT critically contributes to the neurobiological responses to ECS."*

## 2.4. Elucidating the Peripheral Signals that Regulate Neuroplasticity

While serotonin activity in brain function is becoming understood, peripheral signals that might promote neurogenesis or cause the pro-mitotic effect of physical activity are less explored but have remarkable clinical potential. In the DG, soluble factors released by surrounding niches such as the vasculature participate in modulation of the proliferative phase, and are also abundant in blood circulation. To mechanistically link vascular biology with the neurologic effects of serotonin, running-induced cell proliferation was studied in transgenic animal models for 1) the renin-angiotensin system (RAS) and for 2) vasoactive kinin peptides (e.g. bradykinin). Both the Ang-converting enzyme (ACE) 2–the principal regulatory enzyme of the RAS–and bradykinin, important mediators of cardiovascular homeostasis, are expressed in regions of the adult brain, particularly in the hippocampus (Chen *et al*, 2000; Doobay *et al*, 2007; Shughrue *et al*, 2003).

Two studies below investigate the role of 1) ACE2 in serotonin levels, and 2) ACE2 and bradykinin B2 receptor (B2R) and B1 receptor (B1R) in physical exercise and adult neurogenesis.

### 2.4.1. ACE2 as Novel Factor in Serotonin Metabolism and Neurogenesis

As the principal enzyme of the main humoral system in the body, ACE2 regulates cardiovascular homeostasis by controlling the transition from Ang II to Ang-(1-7) which causes vasodilation and anti-inflammation (Santos *et al*, 2013). Recent studies attribute RAS-independent functions to ACE2 as it also plays a role in the absorption of dietary neutral amino acids in the gut, e.g. of L-tryptophan (Trp) (Singer *et al*, 2012), the serotonin precursor. Given that altered Trp levels affect serotonin synthesis, the ACE2 enzyme could modulate brain Trp, and serotonin levels, that indirectly affect neurogenesis.

The following abstract is from the original research article:

“**Klempin F**, Mosienko V, Matthes S, Villela DC, Todiras M, Penninger JM, Bader M, Santos RAS, Alenina N (Depletion of Angiotensin-converting enzyme 2 reduces brain serotonin and

impairs the running-induced neurogenic response. *Cell Mol Life Sci.* 2018 Oct;75(19):3625-3634; doi: https://doi.org/10.1007/s00018-018-2815-y

> *"Physical exercise induces cell proliferation in the adult hippocampus in rodents. Serotonin (5-HT) and angiotensin (Ang) II are important mediators of the pro-mitotic effect of physical activity. Here, we examine precursor cells in the adult brain of mice lacking angiotensin-converting enzyme (ACE) 2, and explore the effect of an acute running stimulus on neurogenesis. ACE2 metabolizes Ang II to Ang-(1-7) and is essential for the intestinal uptake of tryptophan (Trp), the 5-HT precursor. In ACE2-deficient mice, we observed a decrease in brain 5-HT levels and no increase in the number of BrdU-positive cells following exercise. Targeting the Ang II/AT1 axis by blocking the receptor, or experimentally increasing Trp/5-HT levels in the brain of ACE2-deficient mice, did not rescue the running-induced effect. Furthermore, mice lacking the Ang-(1-7) receptor, Mas, presented a normal neurogenic response to exercise. Our results identify ACE2 as a novel factor required for exercise-dependent modulation of adult neurogenesis and essential for 5-HT metabolism."*

### 2.4.2. Bradykinin as Neuropeptide in Activity-Dependent Cell Proliferation

Vasoactive kinin peptides regulate blood pressure and control inflammatory processes by primarily causing endogenous vasodilation. Recent studies show that they also function in the brain, adding novel actions to kinins as neuropeptides. Particularly, bradykinin is involved in glutamate signaling in spinal cord (Kohno *et al*, 2008), and in cell proliferation and fate choice towards neurons *in vitro* (Nascimento *et al*, 2015; Trujillo *et al*, 2012). In disease models, B1R and B2R have been shown to participate in the development of temporal lobe epilepsy (Perosa *et al*, 2007; Silva *et al*, 2008), while in ischemia, B2R is crucial for long-term facilitation of angiogenesis (Xia *et al*, 2006).

In the naïve mouse brain, kinin receptor expression as well as adult neurogenesis had not been described. Taking advantage of mice depleted in B1R, B2R, or both receptors, this study examines the role of kinins as possible neuropeptides in the unchallenged brain, and following physical exercise.

The following abstract is from the original research article:

"Wasinski F, Oliveira Batista R, Bader M, Araujo R, **Klempin F** (Bradykinin B2 receptor is important in running-induced neurogenesis in the adult mouse hippocampus *Brain Struct Funct.* 2018 Nov;223(8):3901-3907; doi: https://doi.org/10.1007/s00429-018-1711-4

> *"Physical exercise is a strong external effector that induces precursor cell proliferation in the adult mouse hippocampus. Research into mechanisms has focused on central changes within the hippocampus and we have established that serotonin is the signaling factor that transduces physical activity into adult neurogenesis. Less focus has been given on potential peripheral signals that may cause pro-mitotic running effects. Vasoactive kinin peptides are important for blood pressure regulation and inflammatory processes to maintain cardiovascular homeostasis. Acting via the two receptors termed B1 (B1R) and B2R, the peptides also function in the brain. In particular, studies attribute B2R*

*a role in cell proliferation and differentiation into neurons in vitro. Here, we determined B1R and B2R mRNA expression levels in the adult mouse hippocampus and prefrontal cortex in vivo, and in response to running exercise. Using mice depleted in either or both receptors, B1-knockout (KO), B2KO and B1/2KO we observed changes in running performance overnight and in running distances. However, voluntary exercise led to the known pro-mitotic effect in the dentate gyrus of B1KO mice while it was attenuated in B2KO accompanied by an increase in microglia cells. Our data identify B2R as an important factor in running-induced precursor cell proliferation."*

# 3. Discussion

Stem cells hold promises to cure neuro-psychiatric diseases. With the capacity to continuously generate new neurons in the adult, NSCs of the DG constitute an exciting endogenous source of multipotent cells that can be deployed to define new drug targets. Stem/progenitor cells are responsive to neural activity as well as environmental intervention. Dissecting NSC behavior in their particular neurogenic niche, and examining how the various signaling factors direct lineage progression is crucial in designing cell-specific therapeutics or to repair damaged brain tissue. My research on 1) ectopic-induced neurogenesis using a single transcription factor, 2) activity-dependent changes in neuroplasticity, and 3) elucidating the role of serotonin and novel peripheral cues, contributes to the understanding of how adult neurogenesis is regulated. In addition, my work clearly shows the importance of serotonin as a key player in antidepressant action. Serotonin not only modulates neuron replacement but also induces additive stimuli that are important for the healthy brain and in drug action.

In the decades after the discovery of adult neurogenesis, numerous studies have investigated principal biological processes, and the contribution of newly generated cells to brain plasticity under physiological conditions (reviewed in Toda *et al*, 2019). Recent studies aim to elucidate the role of adult-born neurons in the diseased brain. Accumulating evidence from rodent models reveals that decreased neurogenesis strongly correlates with impaired cognitive performance and mood disorders. Thereby, changes in cell survival or cell death often co-occur with deregulated neurotrophic and neurotransmitter signaling. In turn, physical exercise and antidepressant treatment upregulate intrinsic factors, cell proliferation and differentiation in animal models; and lead to clinical improvement in patients. This is possible since immature and mature neurons in the rodent DG differ from adult granule cells in their electrophysiological

properties and transient expression pattern (Toni and Schinder, 2015), which makes them preferably affective to stimuli.

Physical exercise is a very strong and extensively studied external stimulus promoting neuroplasticity. Voluntary running increases the number of precursor cells in the SGZ, but also enhances cell proliferation in the newly discovered stem cell niche of the hypothalamus (Klein *et al*, 2019) and the number of Neuron-glia2 (NG2) precursor cells in the amygdala (Ehninger *et al*, 2011). Expanding the pool of glial cells may be particularly important in demyelinating diseases, while enhancing neurogenesis is anticipated following stroke, or in neurodegenerative and psychiatric disorders. My research established that the neurogenic regulatory capacity of physical exercise is mediated through central serotonin (Klempin *et al*, 2013). Furthermore, circulatory factors, e.g. ACE2 or bradykinin, participate in the pro-mitotic effect of running (Klempin *et al*, 2018; Wasinski *et al*, 2018). These discoveries point to a confluence of signals whereby physical activity co-regulates the vascular and neurogenic homeostasis of the brain. In particular, the newly identified pathways have potential as a target for modulation of neuroplasticity alone or to augment physical exercise in the treatment of mood disorders. In humans, both body and brain benefit from activity, and recent studies sought to identify molecules that mimic the system level effects of exercise in the brain. Indeed, factors involved in energy metabolism secreted by skeletal muscle, or dietary supplements, are capable to stimulate cellular targets in the DG (Agudelo *et al*, 2014; Kobilo *et al*, 2011; Moon *et al*, 2016). Furthermore, central regulation alone can yield exercise-like effects ameliorating cognition in an animal model of AD, e.g. combined elevation of type-2b/3 cell numbers and BDNF levels; thereby changes in brain plasticity were mediated by cell-autonomous, autocrine action of BDNF (Choi *et al*, 2018).

My studies of genetically modified mouse models support the importance of brain serotonin in adult neurogenesis, and contribute to the understanding of linked mechanisms implicated in the etiology of depression, e.g. BDNF signaling (Kronenberg *et al*, 2015; Kronenberg *et al*, 2018). Specifically, brain serotonin and BDNF interact to maintain homeostasis in that loss of the neurotransmitter promotes neurotrophic signaling in the

hippocampus. However, using developmental mouse models with altered serotonin levels alone one cannot discriminate between compensatory processes provoked by life-long depletion and phenotypes induced by serotonin *per se*. My current work employs new genetic tools where serotonin levels can be acutely depleted, or repeatedly stimulated, which recapitulate the human condition more adequately (*Tph2*$^{Tph2\text{-}iCre}$ conditional *Tph2*-knockout mouse and *TetO-shTph2* inducible transgenic rats). Potentially, acute changes in behavior, neurogenesis, or BDNF levels following impaired serotonin signaling can be explored, in addition to acute relapses such as those that may follow cessation of SSRIs in patients. This work will yield further important insights into the role of serotonin in the etiology and pathogenesis of affective syndromes and depression that might aid the development of new therapeutic targets.

Psychiatric disorders are polygenic, induced by many different genetic variants (reviewed in Sullivan and Geschwind, 2019). Depression, in particular, is a comorbidity trait also observed in vascular diseases such as dementia, stroke, and myocardial infarction. To innovate and improve pharmacotherapy, the discovery of pathways that regulate cell birth, and the mechanistic link of physical activity and neural circuits that control neurogenesis in normal brain and disease is of importance. Studying transgenic animals is one approach; by using mouse models of the serotonin system as well as of peptide hormones of the vasculature, my data reveal a correlation between system level effects and neurobiology. Newly developed technologies embrace optogenetics and single cell sequencing. Optogenetics, in particular, allows the activation of brain circuits. Using another transgenic model of serotonin signaling, Tph2-ChR2-eYFP mice, enables 1) to observe projections and wiring from raphe nuclei to DG, and 2) recordings from granule cells in the hippocampus that might indicate direct afferent targets from serotonin neurons, and a potential functional interaction between the neurotransmitter and stem/progenitor cells. Furthermore, the optogenetic setup can be used to mimic physical exercise by continues stimulation of the above circuitry. Imaging and anatomical reconstruction of serotonin fiber pathways will reveal a subset of varicose innervation that specifically regulates the neurogenic niche.

Novel methods to define mechanisms and treatment strategies include the generation of neurons *de novo* by directed lineage progression of endogenous stem cells together with metabolic reprogramming (Gascon *et al*, 2016; Klempin *et al*, 2017) or cell fate respecification *in vitro*. My research showed that selectively modifying transcriptional cues in progenitor cells of the DG is sufficient to induce a cell fate switch (Klempin *et al*, 2012). Using iPSCs to achieve a specific phenotype could be used as cell repair strategy in neurodegenerative disorders such as Parkinson's disease, where dopaminergic neurons are lost. In rather dispersed disorders such as AD, where neuron loss is a consequence of pathogenic proteins, or in trauma and upon injury, restoring brain tissue beyond one particular cell phenotype is required. Recently, human-iPSC-based approaches have been developed to distinguish and model the patient-specific complexity of major depression (reviewed in Hoffman *et al*, 2019). Therefore, aiming at cellular and plasticity-regulating genes together with local cues and signaling factors as therapeutic strategy might be effective to improve mood.

Adult neurogenesis critically contributes to brain plasticity. My studies have shown that targeting neurogenesis in rodents is an important mechanism of antidepressant action. Adult-born neurons might also promote human health. However, identifying the role of neuroplasticity in the adult human brain and the relevance in disease has been challenging. Although numerous studies demonstrate that the mammalian brain has the capacity to continuously generate new neurons, primarily shown by $^{3}$H-thymidine labeling and different markers of lineage progression, there are methodological limitations to the detection of newborn cells in the rather large human brain (Kempermann *et al*, 2018). Besides, a recent study casts doubt on whether neurogenesis persists in the adult human hippocampus. Using post-mortem tissue, Alvarez-Buylla and colleagues were unable to detect newborn neurons in the DG of young-adult and adult patients (Sorrells *et al*, 2018). In contrast, subsequent publications by others are in support of the existence of human neurogenesis in aging (Boldrini *et al*, 2018; Moreno-Jiménez *et al*, 2019; Tobin *et al*, 2019). They have combined human brain samples obtained under highly standardized conditions with innovative tissue processing and eloquent control experiments. The studies have identified a high number of neural progenitor cells (Boldrini *et al*, 2018; Tobin *et al*, 2019) and immature neurons in healthy brain tissue compared with pathological samples,

where numbers progressively declined (Moreno-Jiménez *et al*, 2019). The studies for vs. against human neurogenesis disagree in their conclusions based on the (non)detection of marker expression. The discrepancy is most likely due to methodological differences in tissue processing, duration of fixation, fixative and use of anatomical references that define visibility and detection of neuronal lineage markers at different ages and time points. Future research requires the incorporation of more and novel markers of the neural lineage recently established in rodent models. Nonetheless, the latest evidences suggest progenitor cells persist in the adult human brain that play a role in neuroplasticity; yet, it needs to be determined whether they contribute to repair as well. My research adds to the understanding of antidepressant action, and especially demonstrates the mechanistically linked effort of serotonin, the vasculature and physical activity to regulate cell birth. This is not only important for cognitive improvement or treatment of mood disorders, but also relevant in ischemia as response to ectopic challenges. In most parts of the brain, neurogenesis lacks a niche because environmental signals are shut off. Knowledge gained from dissecting NSC behavior and signaling pathways in the defined neurogenic niche microenvironment could potentially be applied to the entire brain and overcome the restricted capacity of the adult brain for regeneration.

# 4. Summary

Self-repair of the adult brain is limited – most diseases lack effective therapy. In order to better understand how a regenerative response can be achieved, studying mechanisms shaping the neurogenic niche, from environmental factors to intrinsic signaling, is of significance. My work highlights the enormous plasticity of the CNS and the crucial role of serotonin in affecting the behavior of neural stem/progenitor cells. It allows important insights into antidepressant strategies that involve physical activity, adult neurogenesis, BDNF, and signals of the vascular niche. Future research will have to elucidate the systemic cues and targets that regulate neuroplasticity and how they become deregulated in disease. It remains to be seen how they will contribute to the development of novel therapies and biomarkers for cognitive disorders.

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# Erklärung

§ 4 Abs. 3 (k) der HabOMed der Charité

Hiermit erkläre ich, dass

- ich weder früher noch gleichzeitig ein Habilitationsverfahren durchgeführt oder angemeldet habe
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Ich erkläre ferner, dass mir die Satzung der Charité–Universitätsmedizin Berlin zur Sicherung Guter Wissenschaftlicher Praxis bekannt ist und ich mich zur Einhaltung dieser Satzung verpflichte.

16.10.2019

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