Neurogenesis within the adult hippocampus under physiological conditions and in depression

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Abstract

Adult neurogenesis can only be observed in some specific brain regions. One of these areas is the dentate gyrus of the hippocampal formation. The progenitor cells located in the subgranular layer of the dentate gyrus proliferate, differentiate, and give rise to young neurons that can become integrated into existing neuronal circuits. Under physiological conditions, hippocampal neurogenesis is linked to hippocampal-dependent learning, whereas deficits in adult hippocampal neurogenesis have been shown to correlate with disturbances in spatial learning and memory. This review summarizes the phenomenon of adult hippocampal neurogenesis and the use of suitable markers for the investigation of adult hippocampal neurogenesis. In addition, we focused on the disturbances in neurogenesis that can be seen in depression. Interestingly, several antidepressants have been found to be capable of increasing the rate of hippocampal neurogenesis. Based on that, it can be speculated that factors, which directly or indirectly increase the rate of hippocampal neurogenesis, may be helpful in the treatment of depression.

Key Words: hippocampus; dentate gyrus; learning; depression; aging; environment
Abbreviations: SVZ, subventricular zone; NeuroD, neurogenic differentiation; FGF, fibroblast growth factor; BDNF, brain-derived neurotrophic factor.

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INTRODUCTION

Neurogenesis has long been believed only to occur during brain development. In the 1960s, Altman and Das provided the first evidence that new cells can be generated in the postnatal brain[1-2]. Nowadays it is widely accepted that ongoing adult neurogenesis can be observed in several brain regions including:

(i) the subventricular zone (SVZ): The newly born cells of the SVZ migrate and differentiate into neurons within the olfactory bulb[3]. The newly generated neuronal cells in the SVZ migrate over a long distance to the olfactory bulbs through the rostral migratory stream and differentiate into interneurons at their final destination. These newly generated neuronal cells in the olfactory bulbs establish synaptic contacts and functional connections with neighboring cells[4-5].

(ii) the subgranular zone of the dentate gyrus: The newly formed cells integrate into the granular layer of the dentate gyrus and start to extend their axons and dendrites into their target areas[6].

(iii) the neocortex[7-8], the piriform cortex[9-10], the subcallosal zone[11], the amygdala[12], the striatum[13-14], and the substantia nigra[15-16]. However, neurogenesis in these areas seems to occur at substantially lower levels or might be induced under non-physiological conditions.

The phenomenon of adult hippocampal neurogenesis and the use of suitable markers for the investigation of adult hippocampal neurogenesis are reviewed. In the review, we analyze the current knowledge on the effects of depression upon hippocampal neurogenesis and propose that growth factors may not only play important roles for adult hippocampal neurogenesis but also for the beneficial effects of chronic antidepressant treatments on adult neurogenesis within the hippocampus.

THE HIPPOCAMPUS

In humans, the hippocampus is located within the temporal lobe. The hippocampal formation is composed of the Cornu ammonis (areas CA1–CA3), the dentate gyrus and the subicular complex[17]. The hippocampus has a three-laminar composition. In the areas CA1–CA3, these layers are called: (i) stratum oriens, (ii) stratum pyramidale (which consist of cell bodies of the pyramidal neurons) and (iii) the stratum radiatum/lacunosum moleculare. The dentate gyrus has also a laminar organization (stratum moleculare, stratum

granulosum (composed of the cell bodies of the granule cells) and polymorph layer). A substructure belonging to the dentate gyrus is the subgranular zone. This small cell layer is located at the basis of the stratum granulosum and cells located in this substructure are capable of adult neurogenesis. Different parts of the hippocampus (dentate gyrus, CA3, CA1) are interconnected by the so-called tri-synaptic circuit[18-20], which plays a fundamental role in hippocampal learning and memory. The connections within the hippocampus are illustrated in Figure 1.
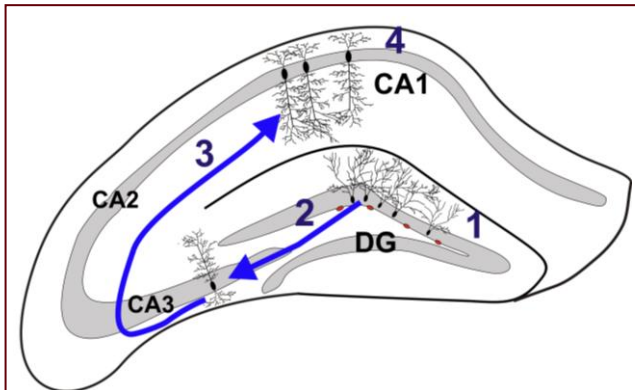

Figure 1 Schematic representation of the connections within the hippocampus (section of a rodent brain).

1: Fibers from the entorhinal cortex (layers II and III) project *via* the *Tractus perforans* to the dentate gyrus;

2: The dentate gyrus sends fibers toward area CA3 ("mossy fiber" system);

3: Areas CA3 and CA1 are connected *via* the Schaffer collaterals;

4: Area CA1 projects back to the entorhinal cortex (layer IV). These projections can be direct or indirect (*via* the subiculum).

Neurogenic cells, located in the subgranular zone, are shown as red circles.

ADULT NEUROGENESIS WITHIN THE HIPPOCAMPUS

Under physiological conditions, adult neurogenesis within the hippocampus is mainly observed in the subgranular layer of the dentate gyrus. Adult hippocampal neurogenesis is thought to consist of several developmental stages[21-22] that are characterized by morphological distinct cells:

Type 1 cells
Adult hippocampal neurogenesis originates from a cell with functional and morphological characteristics of a glia cell. Type 1 cells are thought to constitute the resident early precursor population. The somata of these cells, located in the subgranular zone, are triangular shaped. These cells extend an apical process towards the molecular layer of the dentate gyrus and sometimes also shorter tangentially orientated processes at the base of

the subgranular zone[23-25]. Cells belonging to type 1 are relatively abundant within the subgranular zone, but it is thought that these cells rarely divide. Type 1 cells express glial fibrillary acidic protein as well as the intermediate filament nestin[25-26].

Type 2 cells
Type 1 cells give rise to fast proliferating intermediate precursors. Most of the expansion of the pool of newly generated cells occurs during the stage of type 2 cells. Type 2 cells are characterized by a small soma, irregular shaped nucleus and short and horizontally orientated processes. Type 2 cells show an overlap in the expression of several glial and neuronal markers. Based on the expression of distinct markers, type 2 cells can be divided into two nestin-positive subpopulations: one negative and one positive for the immature neuronal marker doublecortin. These two different subpopulations were named type-2a and type-2b cells[22].

Type 3 cells
Type 3 stage is a transition phase from the slowly proliferating "neuroblasts" to the postmitotic immature neuron. Under normal conditions, type 3 cells display only little proliferative activity, but under pathophysiological conditions (*e.g.* seizures), they can dramatically increase their proliferative activity[27]. Type 3 cells express no markers of the glial lineage, but markers of the neuronal lineage[28]. Type 3 cells migrate over a short distance into the granular layer. The morphology of the type 3 cells is highly variable, reflecting their developmental transition: the orientation of the processes changes from horizontal to vertical and the processes vary in length and complexity. Exit from the cell cycle occurs at this stage and coincides with the transient expression of the calcium-binding protein calretinin. Concerning the regulation and control of adult neurogenesis, it is important to understand that control means maintenance of a baseline level of neurogenesis, whereas regulation is rather the deviation from that baseline. For detailed information see the recent published review from Gerd Kempermann[29].

MARKERS FOR NEUROGENESIS IN THE HIPPOCAMPUS

Bromodeoxyuridine (BrdU)
The synthetic halogenated pyrimidine analogue BrdU is incorporated into nuclear DNA as bromouracil, replacing thymidine during the S-phase of the cell cycle. Since the introduction of monoclonal antibodies against BrdU[30], an increasing number of immunohistocytochemical techniques have been applied for detecting the exogenous nucleoside incorporated into replicating DNA[31]. A breakthrough for the analysis of adult hippocampal neurogenesis was the use of BrdU-immunohistochemistry[32]. However, it should be kept in mind that BrdU labels all S-phase cells in the adult dentate gyrus; thus, it does not allow distinguishing between newly formed glia cells or neurons. Therefore,

BrdU labeling without the use of additional markers is inappropriate. Due to the fact that BrdU incorporation is a general indicator for cell genesis in the brain, an increase in BrdU-labeled cells must not *per se* indicate the generation of more new neurons[6]. To determine that changes in BrdU-labeling are indeed related to altered neurogenesis, one has to combine this labeling with other markers that label newly formed neurons at later stages in the time-course of neurogenesis. Furthermore, problems of dilution, over- and underestimation have to be taken into account. In addition, the application mode (number of injections, concentration of BrdU) and the time-course by which the analysis takes place are important factors that also should be taken into account[33]. BrdU is mainly applied by intraperitoneal injection. Thus, BrdU has to cross the blood-brain barrier and based on that, changes in the integrity of the blood-brain barrier can influence the numbers of BrdU-positive cells[33].

Ki-67 and phosphohistone H3
Both markers are well-suited to detect dividing cells within the hippocampus.
The name Ki-67 is derived from the city of origin (Kiel, Germany) and the number of the original clone in a 96-well plate[34]. Ki-67 is expressed in all phases of the cell cycle (G_1, G_2, S and M) except the resting phase (G_0) and at the beginning of the G_1 phase[35]. Due to its short half-life of about 1 hour, it is rarely detectable in cells in the G_0 phase[35]. Phosphohistone H3 is a part of the histone octamer. The phosphorylated form of phosphohistone H3 is present during the late G2 phase and in the M phase of cell division[36-37]. Since metaphase chromosomes are always intensely phosphorylated, it could be speculated that the phosphorylation of histone 3 is a tool of the cell cycle to mark chromosomes that are ready to go further in the cell cycle[38]. Both markers are widely used to identify proliferating and mitotic cells in the hippocampus (Figure 2A) and in contrast to BrdU, Ki-76 and phosphohistone H3 can directly be detected by means of immunohistochemistry, since they are intrinsically expressed.
Since the mere measurement of proliferation is not a good prognosis of neurogenesis as a process[29], a more detailed analysis by using markers for later steps of neurogenesis is required.

Neurogenic differentiation (NeuroD)
The basic helix-loop-helix protein NeuroD represents a transcription factor expressed at later stages of neuronal commitment[39]. NeuroD is expressed during neurogenesis in the adult dentate gyrus[40] and NeuroD-positive cells can be found in the subgranular zone and inner granule cell layer[41-42]. Furthermore, NeuroD expression is found in PSA-NCAM-positive cells (see below) within the dentate gyrus, but NeuroD expression precedes that of PSA-NCAM[43]. Thus, NeuroD is a marker for the early cells of the neuronal lineage and therefore can be used to identify early mitotic active neuronal cells in the dentate gyrus.

Polysialylated embryonic form of the neural cell adhesion molecule (PSA-NCAM) and doublecortin
PSA-NCAM is highly expressed by newly generated and developing granule cells in the adult dentate gyrus[44]. The attachment of the polysialic acid with its high negative charge and large hydrate volume leads to a weakening of the adhesion forces; however, the functional significance of polysialic acid on NCAM in adult neurogenic zones is incompletely understood[45]. doublecortin (Figure 2B) is a brain-specific microtubule-associated protein that is expressed by migrating neuroblasts and young neurons[46-47].
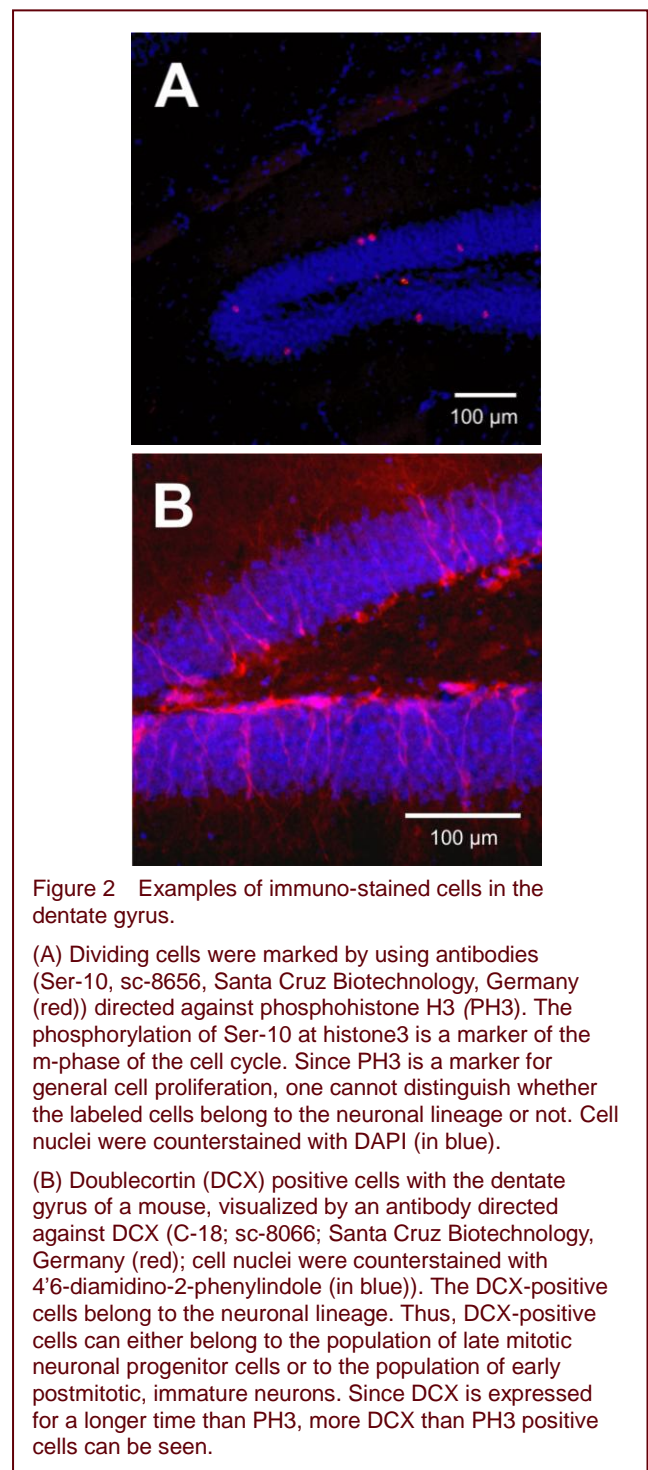

Figure 2 Examples of immuno-stained cells in the dentate gyrus.

(A) Dividing cells were marked by using antibodies (Ser-10, sc-8656, Santa Cruz Biotechnology, Germany (red)) directed against phosphohistone H3 *(*PH3). The phosphorylation of Ser-10 at histone3 is a marker of the m-phase of the cell cycle. Since PH3 is a marker for general cell proliferation, one cannot distinguish whether the labeled cells belong to the neuronal lineage or not. Cell nuclei were counterstained with DAPI (in blue).

(B) Doublecortin (DCX) positive cells with the dentate gyrus of a mouse, visualized by an antibody directed against DCX (C-18; sc-8066; Santa Cruz Biotechnology, Germany (red); cell nuclei were counterstained with 4'6-diamidino-2-phenylindole (in blue)). The DCX-positive cells belong to the neuronal lineage. Thus, DCX-positive cells can either belong to the population of late mitotic neuronal progenitor cells or to the population of early postmitotic, immature neurons. Since DCX is expressed for a longer time than PH3, more DCX than PH3 positive cells can be seen.

In 1998, it has been discovered that mutation in the human X-linked gene doublecortin causes defects in the cortical layering, known as "double cortex" syndrome, in females, whereas males show X-linked lissencephaly[48-49]. A central phase of neurogenesis is associated with the expression of PSA-NCAM and doublecortin. This phase ranges from the progenitor stage to the stage, during which the newly generated cells extend their dendrites and axons to establish functional connections[50]. PSA-NCAM or doublecortin positive cells are also positive for NeuroD and there is a transient co-expression of these markers with NeuN, a marker for mature neuronal cells[41, 51-52], indicating that PSA-NCAM and doublecortin are markers for the neuronal lineage in the adult dentate gyrus.

FUNCTIONAL HIPPOCAMPAL NEUROGENESIS

Neurogenesis within the dentate gyrus occurs throughout postnatal life and is influenced by environment, behavior[53-57] and aging[58-59].
The hippocampus is a brain region capable of structural reorganization. Pre-existing neural circuits within the adult hippocampus can undergo experience-induced changes in dendritic spines[60] and in the rate of hippocampal neurogenesis. Functional neurogenesis seems to have a profound impact upon neuronal plasticity within the hippocampus, since increased neurogenesis within the dentate gyrus is observed in a variety of hippocampus-dependent learning and memory tasks[21, 61-62]. Furthermore, long-term potentiation, a well-characterized form of synaptic plasticity, believed to play a critical role in memory formation, stimulates hippocampal neurogenesis[63]. Deficits in adult hippocampal neurogenesis, however, can lead to defective spatial learning and memory[64].
Since the hippocampus exhibits marked functional decline with aging, it could be speculated that neurogenesis within the aged dentate gyrus is altered. Indeed, it has been shown that neurogenesis is drastically reduced in aged animals, not only in rodents, but also in non-human primates[65]. Neurogenesis therefore seems to be linked to hippocampal functions and an age-related decline in hippocampal functions seems to be accompanied by a reduction in neurogenesis. When talking about aging, it is worth to consider that not aging *per se* is the "regulator" of neurogenesis[29] but rather one of the global key determinants that (in concert with other regulators) can influence both the baseline level and the regulation of adult neurogenesis[66]. From the factors that cause a decline in neurogenesis during aging, several neuropeptides and transmitters are known to be involved. For example, modified expression of neurotrophic factors[58], changed neurotransmitter release[67-70], as well as increased glucocorticoid levels have been found to contribute to the effect of aging on adult neurogenesis[66].

Therefore, the decline in hippocampal neurogenesis may represent a side-product of aging. Given that neurogenesis occurs throughout postnatal life, one would expect that the dentate gyrus increases in size during adulthood and that the number of granule cells is increased in aged as compared to adult animals. However, granule cell number of the dentate gyrus do not increase with age[71-73], indicating that proliferation is balanced by cell death. Thus, not the addition of new neurons into the dentate gyrus seemed to be linked to hippocampal functions, but the rate of the turnover of granule cells within the dentate gyrus.
Neurogenesis is not only influenced by intrinsic mechanisms but is altered by external stimuli. For example, neurogenesis within the dentate gyrus is increase in mice that were housed in an enriched environment[54]. Enriched environment not only increases neurogenesis in the dentate gyrus, but also improves spatial memory[74] and levels of neurotrophins[75]. Interestingly, there are data suggesting that the newly generated neurons participate in the memory improvement induced by the enrichment[76]. Environmental enrichment typically consists of many components, including increased social interaction and more physical activity. Concerning the increased social interactions, it has been found that social environment has an impact upon adult neurogenesis, since rats reared in isolation have less newborn cells in the dentate gyrus than rats housed in groups[77]. Thus, social environment can increase neurogenesis. Interaction with social partners, however, can lead to the formation of dominance hierarchies. The position in a dominance hierarchy does not influence cell proliferation within the dentate gyrus, but dominant male rats have more new neurons as compared with male subordinates[78]. Based on these results, it can be concluded that social environment is capable of increasing cell proliferation in the dentate gyrus and the position within the dominance hierarchy has an effect upon the neuronal lineage. Since environmental enrichment allows more physical activity, it also has been tested whether voluntary running influences adult neurogenesis. The obtained data demonstrate that voluntary exercise increases the number of BrdU-positive cells in the adult murine dentate gyrus[79] and the levels of the brain-derived neurotrophic factor (BDNF)[75]. These data indicate that both components of enriched environment (social interactions and physical activity) contribute to the beneficial effects upon adult neurogenesis. Furthermore, a dietary restriction feeding regimen has been found to increase the numbers of newly generated cells in the murine dentate gyrus, along with an increase in the expression of neurotrophins[80].

HIPPOCAMPAL NEUROGENESIS AND DEPRESSION

Depression is a disorder of the representation and

regulation of mood and emotion. Mechanisms underlying the etiology of depression are complex and still poorly understood[81]. Depression has a profound impact upon several brain structures, as e.g. the hippocampal formation[82]. In numerous magnetic resonance imaging studies, the hippocampal volumetric differences between depressed and healthy subjects have been analyzed. Although there are some inconsistencies among the different studies, a smaller hippocampal volume in depressed patients seems to be related to the pathophysiology of the disease[83]. An interesting aspect of antidepressant treatment is that it is capable of blocking or reversing hippocampal atrophy that is observed in patients with depression[84].

It has been reported that stress paradigms, as well as some animal models of depression, result in a decrease in hippocampal volume and neurogenesis[85-86]. In this context it is of interest to note that that chronic antidepressant treatment is not only effective in the treatment of depression, but also up-regulates adult neurogenesis within the hippocampus[86-87]. Current evidence suggest that adult hippocampal neurogenesis may not be a major contributor to the development of depression, but is required for some of the effects of antidepressants[88-89]. This would suggest that there is a link between the chronic treatment with antidepressants and neurogenesis. Such a link may be the effect of several antidepressants on neurotrophins. Antidepressants stimulate the production and signaling of plasticity-related proteins such as neurotrophins[90]. The neurotrophin BDNF, for example, promotes neuronal differentiation, survival during early development, adult neurogenesis, and neural plasticity[91]. Thus, treatment with antidepressant may, through enhanced BDNF signaling, improve neurogenesis. Aside from neurotrophins also other growth factors seemed to be important in this context. By comparing depressed subjects, treated with antidepressants, with untreated depressed subjects, it was demonstrated that depression-induced changes in fibroblast growth factor (FGF) transcripts, including FGF-2, were attenuated by antidepressant treatment[92]. Moreover, it has been shown that FGF-2 injection into the lateral ventricle induces antidepressant-like effects[93-94] and that mice lacking FGF-2 display reduced hippocampal neurogenesis[95]. Thus, FGF-2 may also constitute both, a potential partner interacting with several antidepressants and a factor influencing adult hippocampal neurogenesis. Based on these results it can be suggested that neurogenesis is affected by depression and that chronic antidepressant treatment can have beneficial effects on hippocampal neurogenesis. These results further hint that chronic treatment with antidepressants has an impact upon various growth factor systems and that these different growth factors, at least in part, may be responsible for the beneficial effects of several antidepressants. However, these assumptions are based on animal models of depression. Concerning depression in humans, it would be important to show that there is also a link between depression and neurogenesis. Whether antidepressant interventions produce similar alterations in the human brain and whether stimulation of adult neurogenesis within the human hippocampus may have beneficial effects in the treatment of depression is still unknown.

Data from animal studies have shown that neurogenesis can be increased by dietary restriction as well as by enriched environment. Both conditions not only increase the rate of hippocampal neurogenesis but also the levels of BDNF. Thus, physical exercise and environmental enrichment may have beneficial effects in the treatment of depression. Along this line, it has been demonstrated that exposure to enriched environment ameliorates depressive symptoms in chronically stressed rats[96] as well as in rats exposed to juvenile stress[97].

CONCLUSION/PERSPECTIVE

New neurons generated in the dentate gyrus of the adult hippocampus play an essential role in certain forms of hippocampus-dependent learning tasks. Getting insight in the role of adult hippocampal neurogenesis in learning and memory could have important clinical implications. Stimulation of neurogenesis within the dentate gyrus could have the capacity to recover cognitive functions and might therefore be beneficial for the treatment of neurodegenerative (*e.g.* Alzheimer's disease) and/or mental disorders (*e.g.* depression). Environmental enrichment, as well as pharmacological manipulations, such as treatment with certain antidepressants, can be helpful in promoting neurogenesis. Depression is accompanied by a decline in neurogenesis and chronic treatment with antidepressants can increase the rate of neurogenesis. It can be speculated that the stimulating effects upon neurogenesis of diverse antidepressants are mediated by the activation of growth factors. Environmental enrichment and physical exercise increase the rate of neurogenesis within the dentate gyrus, increase the levels of growth factors and ameliorate depressive symptoms. Environmental enrichment and physical exercise may therefore provide new therapeutic strategies for the treatment of depression or of illnesses that are accompanied by reductions in the rates adult neurogenesis.

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Brief research background of the authors and their laboratory
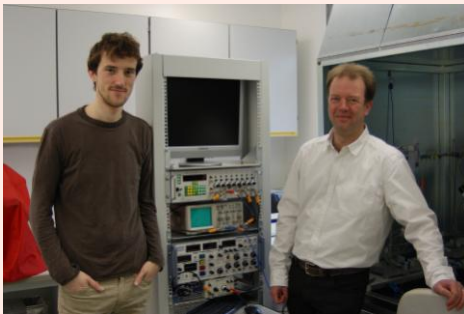
Martin Dokter (left)
Oliver von Bohlen und Halbach (right)

The authors' group mainly works on mechanisms related to neuronal plasticity within the limbic system, by focusing on the hippocampal formation and the amygdala. They are interested in getting insight in the roles of growth factors (*e.g.* neurotrophins and fibroblast growth factors) in the adult limbic system under physiological as well as psychopathological conditions (*e.g.* depression, mental retardation). They mainly use animal models (transgenic rodents) and morphological (dendritic spines, adult neurogenesis) as well as electrophysiological correlates (*e.g.* long-term potentiation) of neuronal plasticity for analyzing the effects of growth factors and their receptors upon neuronal plasticity in adult and aged rodents.

Recent Publications of Oliver von Bohlen und Halbach

1. Werner S, Unsicker K, von Bohlen und Halbach O. Fibroblast growth factor-2 deficiency causes defects in adult hippocampal neurogenesis, which are not rescued by exogenous fibroblast growth factor-2. J Neurosci Res. 2011;89(10):1605-1617.

2. von Bohlen und Halbach O. Immunohistological markers for proliferative events, gliogenesis, and neurogenesis within the adult hippocampus. Cell Tissue Res. 2011;345(1):1-19.

3. Zschenderlein C, Gebhardt C, von Bohlen und Halbach O, et al. Capsaicin-induced changes in LTP in the lateral amygdala are mediated by TRPV1. PLoS One. 2011;6(1):e16116.

4. Schindowski K, von Bohlen und Halbach O, Strelau J, *et al.* Regulation of GDF-15, a distant TGF-β superfamily member, in a mouse model of cerebral ischemia. Cell Tissue Res. 2011;343(2):399-409.

5. von Bohlen Und Halbach O. Dendritic spine abnormalities in mental retardation. Cell Tissue Res. 2010;342(3):317-323.

6. von Bohlen und Halbach O. Involvement of BDNF in age-dependent alterations in the hippocampus. Front Aging Neurosci. 2010;2. pii: 36.

7. Zechel S, Meinhardt A, Unsicker K, von Bohlen und Halbach O. Expression of leucine-rich-repeat-kinase 2 (LRRK2) during embryonic development. Int J Dev Neurosci. 2010;28(5):391-399.

8. Enkel T, Gholizadeh D, von Bohlen Und Halbach O, *et al.* Ambiguous-cue interpretation is biased under stress- and depression-like states in rats. Neuropsychopharmacology. 2010;35(4):1008-1015.