Dissertation

Adult neurogenesis as a potential source for regeneration of structural brain damage – Redirection of neuronal precursor cells from the rostral migratory stream into prefrontal cortex lesions in adult rats

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Jannis Gundelach

Institut für Hirnforschung, Abteilung Neuropharmakologie, Zentrum für Kognitionswissenschaften

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Universität Bremen

1. Gutachter: Prof. Dr. Michael Koch

2. Gutachter: Prof. Dr. Ursula Dicke

Universität Bremen

Jannis Gundelach
Hohentorstr. 80/82, 28199 Bremen

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ERKLÄRUNG

Hiermit erkläre ich, dass ich die Doktorarbeit mit dem Titel:

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List of publications

(*****) indicates publications or manuscripts included in this thesis. Articles have been published or submitted to international scientific journals.

* **Gundelach, J.** & Koch, M. (2018). EndoN treatment allows neuroblasts to leave the rostral migratory stream and migrate towards a lesion within the prefrontal cortex of rats. *PLOS ONE*. (Under revision)

* **Gundelach, J**. & Koch, M. (2018). Redirection of neuroblast migration from the rostral migratory stream into a lesion in the prefrontal cortex of adult rats. *Experimental Brain Research*, *236*(4), 1181–1191. https://doi.org/10.1007/s00221-018-5209-3

Schulz S, **Gundelach J**, Hayn L, Koch M, Svärd HK (2014) Acute Co- Administration of the Cannabinoid Receptor Agonist WIN 55-212,2 does not Influence 3,4-Methylenedioxymetamphetamine (MDMA)-Induced Effects on Effort-Based Decision Making, Locomotion, Food Intake and Body Temperature. Biochem Pharmacol 3:127. doi:10.4172/2167-0501.1000127

Abbreviations

ANOVA	analysis of variance
ApoER2	apolipoprotein E receptor 2
BDNF	brain-derived neurotrophic factor
BR	breathing rate
BrdU	bromdesoxyuridin
CDrev	compound discrimination
CNS	central nervous system
DCX	doublecortin
DR	death receptors
DTI	diffusion tensor imaging
endoN	endoneuraminidase-N
Eph	erythropoietin-producing human hepatocellular receptors
EPI	echo planar imaging
ErbB4	receptor tyrosine-protein kinase erbB-4
FA	fractional anisotropy
Fas	first apoptosis signal receptor
GDNF	glial cell-derived neurotrophic factor
GFAP	glial fibrillary acidic protein
IQR	interquartile range
L-DOPA	L3,4-dihydroxyphenylalanine
MCP-1	monocyte chemoattractant protein-1
mPFC	medial prefrontal cortex
MRI	magnetic resonance imaging
NCAM	neural cell adhesion molecule
NeuN	neuronal nuclei
NMNAT2	nicotinamide mononucleotide adenylyltransferase 2
NRG1 and 2	neuregulin 1 and 2
OB	olfactory bulb
PBS	phosphate buffered saline
PNS	peripheral nervous system
PSA	polysialic acid
RMS	rostral migratory stream

Robo2 and 3 roundabout homolog 2 and 3

ROI region of interest

SCF stem cell factor

SDF-1α stromal cell-derived factor 1 α

SEM standard error of the mean

SVZ subventricular zone

T(D) trace of diffusion

TBI traumatic brain injury

TE echo time

TNF tumor necrosis factor

TR repetition time

TWI trace weighted image

VEGF vascular endothelial growth factor

VLDLR very-low-density-lipoprotein receptor

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1 Introduction

Up until today, no cure for structural brain damage exists. The consequences of neuronal loss are treated symptomatically and rehabilitation measures support the brain's own limited ability to shift functions from the lost tissue to other areas, while the lesioned tissue is considered irreversibly lost. However, two distinct areas of the mammalian brain, one within the hippocampal formation and one around the walls of the lateral ventricles, keep producing neuronal precursor cells throughout life. The neuroblasts generated in these areas follow distinct migration patterns and are generally not available for repair of brain damage. Within the scope of this thesis two different methods are evaluated that enable neuroblasts to leave their path through the forebrain of rats and migrate towards nearby damaged tissue.

1.1 General Motivation

1.1.1 The brain at risk: Multiple paths to structural brain damage

Through the course of a human life our brain is at constant danger of sustaining structural damage. Not only accidents can lead to traumatic brain damage, also neurodegenerative diseases or exposure to certain toxins can cause irreversible loss of brain cells.

Traumatic brain injury (TBI)

TBI is a major health issue worldwide and one of the leading cause of mortality and disability in high income countries (Maas et al. 2008). As such it imposes a severe socioeconomic burden since TBI often affects middle aged individuals causing long term disability (Faul and Coronado 2015). The causes of TBI are manifold and vary depending on factors such as age or occupation. For example, falls are the major cause of TBI in children and elderly, whereas young adults more often encounter TBI in motor vehicle accidents or acts of violence (Taylor et al. 2017). Other risk factors for TBI include contact sports such as American football or boxing (Kulbe and Hall 2017) or exposure to blasts among military personnel (Albanese et al. 2018; Gill et al. 2018).

Similarly, diverse as the causes are the forms of head injuries that lead to TBI: If the *dura mater* has been pierced, either by a foreign object or pieces of the skull, the head

injury is classified as penetrating, which often results in a focal damage to the brain. But also a closed head injury can cause a compression of the underlying cerebral tissue (coup) and the tissue on the opposing side of the brain (contrecoup) (Pudenz and Shelden 1946).

In contrast, a rapid de- or acceleration or a shockwave can cause a diffuse or multifocal injury, which often affects the axonal white matter (Committee on Gulf War and Health 2005).

However, it is not always possible to predict tissue damage by the type of head injury. For example, a focal injury may cause a subarachnoid hemorrhage which in turn often leads to a diffuse brain damage. In fact, an MRI study on patients with moderate or severe head injury found combined focal and diffuse TBI in half of the subjects (Skandsen et al. 2010).

The symptoms of TBI and their recovery generally follow a distinct pattern, although the duration of each stage is highly dependent on the severity of the trauma (Povlishock and Katz 2005): The first state, unconsciousness, starts immediately after the injury and may last from seconds to weeks. It is followed by a phase of emerging consciousness and confusion, usually combined with anterograde amnesia, before the phase of post-confusional restoration of cognitive functions starts. Notably, this pattern of restoration is less recognizable after focal TBI, in which the symptoms are dependent on the area affected, the size of the lesion, and if the impact occurred bilaterally or not (Povlishock and Katz 2005).

The course of a TBI can be divided into two phases, the primary phase, which is a direct consequence of the mechanical forces on the cerebral tissue, and the secondary injury, which describes the cellular processes that ultimately lead to cell death, but also includes cell loss due to hypoglycemia, hypoxic conditions or raised intracranial pressure (Andriessen et al. 2010).

Neurodegenerative diseases

The other danger to the human brain lies in numerous neurodegenerative diseases. Especially in today's aging society, these disorders become a growing burden. The most common example for pathologic neurodegeneration is dementia, affecting about 46 million people worldwide (Vos et al. 2016). The most predominant cause of dementia is

Alzheimer's disease (Burns and Iliffe 2009), which leads to atrophy of the hippocampus, the amygdala and the neocortex. Notably, the progressive degeneration of the cortical tissue follows a distinct pattern and spares the motor cortex until late stages of the disease (Pini et al. 2016)

In Parkinson's disease, on the other hand, a loss of dopaminergic cells in the substantia nigra leads to a number of specific motor symptoms, such as tremor, rigidity, and slowness of movement, even at early stages of the disease (Clarke 2007). The disease is often accompanied by cognitive and psychiatric disorders such as slowed cognitive processing, depression and anxiety, apathy, and hallucinations (Jankovic 2008). At later stages, patients have an increased risk of developing dementia (Aarsland et al. 2001).

Another example for a rather commonly known neurodegenerative disorder is Huntington's disease. In contrast to the two aforementioned examples, the cause of Huntington's has been identified as an inherited, genetic disorder. Extended cytosine-adenine-guanine triplet repeats in the Huntingtin gene lead to the expression of a mutant form of the eponymous huntingtin protein in neurons throughout the central nervous system (Novak and Tabrizi 2010). The exact mechanism, through which the protein causes neurodegeneration, is not known (Bates et al. 2015), but patients suffer from loss of GABAergic neurons in the striatum and widespread neuronal cell loss throughout the whole brain at later stages of the disease (Dayalu and Albin 2015). The symptoms include progressive motor disorder and progressive cognitive dysfunction which leads to dementia, but also, predominantly at later stages, psychiatric disturbances such as depression, anxiety, apathy, and obsessive compulsive behavior (Dayalu and Albin 2015). Huntington's disease leads to death 15 to 20 years after diagnosis (Roos et al. 1993).

The brief description of some neurodegenerative diseases above is intended to demonstrate the diversity of these disorders. While most of the processes rather start at an advanced age, others, such as Huntington's, commonly develop symptoms in patients in their thirties (Dayalu and Albin 2015). Similarly different are the temporal and spatial patterns of neurodegeneration and the consequential symptoms. As mentioned above, patients with Parkinson's disease suffer from motor symptoms early on, while dementia is a symptom typically observed in the late stages. On the other hand, in Alzheimer's disease motor deficits are usually not observed until the late progression of the disease. The common ground of these disorders is the incurable loss of neuronal tissue within

the patient's brain. So far, the treatment is limited to the managing the symptoms for example by application of the catecholamine-precursor L-DOPA (L3,4-dihydroxyphenylalanine) to counteract the lack of dopamine in Parkinson patients (Olanow et al. 2004). Finding a technique that intervenes with the specific pathology of the diseases is topic of ongoing research and has not been achieved so far (Stocchetti et al. 2015; Cummings 2017). However, even if such a treatment has been developed, a method to repopulate the impaired brain areas with new neurons would be highly desirable.

1.1.2 Mechanisms of cell death

Although the final outcome of TBI, ischemia, or neurodegenerative disease, the irreversible loss of neurons, is identical, the underlying signaling cascades might be very different and will be characterized here briefly.

Apoptosis

Apoptosis, or programmed cell-death, is the mechanism of cell loss in multiple pathological processes, but also provides an important physiological mechanism during development and in processes related to immune functions or cell turnover (Elmore 2007). It is an evolutionary conserved function but the cascades involved in the regulation of apoptosis are more complex in higher developed animals, offering multiple activators and inhibitors and several backup systems (Fuchs and Steller 2011). Apoptosis is generally initiated by activation of the so called death receptors of the tumor necrosis factor (TNF) receptor superfamily. These receptors include TNF-α, Fas (first apoptosis signal receptor), and the DR (death receptors) 3, 4, and 5 (Elmore 2007). The signaling cascade is further mediated over caspases 8 and 10 which activate caspase 3, but also trigger cytochrome c release from mitochondria. This indirectly activates caspase 3, which in turn triggers cell shrinkage, chromatin condensation, DNA-fragmentation, membrane blebbing, and the following formation of apoptotic bodies. These remains of the cells are then removed by phagocytic cells.

Apart from the activation of death receptors, apoptosis can be triggered by intrinsic signals via the mitochondrial pathway. This is known to happen for example in response to DNA damage, chemotherapeutic agents, serum starvation, and UV radiation (Wang and Youle 2009).

Necrosis and necroptosis

Another, caspase-independent and generally less regulated mechanism of cell death is necrosis. It is characterized by the morphological changes a necrotic cell undergoes: much in contrast to apoptotic cells, here the cell and its organelles, such as the endoplasmic reticulum and mitochondria, swell and rupture, whereas the nucleus stays mostly intact (Nikoletopoulou et al. 2013).

Necrosis is usually associated with severe cell trauma or radiation and is independent of the aforementioned death receptors. However, activation of some specific death receptors, but also members of the pathogen recognition receptors have been shown to induce an alternative, more regulated form of necrosis. This rather recently discovered process has been termed necroptosis and is thought to act as an alternative to apoptosis, if the apoptotic cascade cannot be executed (Nikoletopoulou et al. 2013). This has been demonstrated to happen after inhibition of caspase (Los et al. 2002), but also with low cellular ATP levels (Eguchi et al. 1997).

Autophagy

Autophagy is a crucial process for cell survival, since it removes proteins and remains of turned over cell organelles from the cytoplasm. The targets to be removed are engulfed by autophagosomes, vesicles with double membranes, which then fuse with the lysosome, where the material is degraded by specific acidic hydrolases (Klionsky and Emr 2000). Furthermore, autophagy can protect cells from starvation, as parts of the cell are degraded in order to ensure its survival (Kuma et al. 2004). On the other hand, autophagy is tightly coupled to apoptotic cell death, since pro- and anti-apoptotic factors also up- or down-regulate autophagy (Mizushima et al. 2008).

Especially in neurons autophagic homeostasis is required for cell survival: Since adult neurons do not undergo cell division, they cannot regulate the amount of damaged cell organelles or certain proteins by passing them on to daughter cells and therefore rely on autophagy. Thus, disruption of autophagy is related to multiple neurodegenerative processes (Hara et al. 2006).

In particular, diseases that are characterized by formation of protein aggregates, such as Huntington’s and Parkinson’s disease (and in a more indirect way Alzheimer’s disease), are connected to malfunction of autophagy. However, it is not completely understood

yet, if functioning autophagy would degrade the built up aggregates or rather ensure continuous removal of cytosolic proteins and therefore prevent the protein aggregation in the first place (Mizushima et al. 2008).

Wallerian degeneration

Wallerian degeneration is a mechanism specific to nervous tissue, and is caused by anoxia and ischemia (Stys et al. 1992) or traumatic axonal damage (Pettus et al. 1994). When an axon is severely damaged, the anterograde and retrograde transport mechanisms break down, which leads to the degeneration of the fiber distal to the soma (Waller 1850). The underlying triggers for this process remain unknown, but a study on knock out mice has identified sufficient levels of the enzyme NMNAT2 (Nicotinamide mononucleotide adenylyltransferase 2) as a prerequisite for axonal survival (Gilley and Coleman 2010). After degeneration of the affected fibers, the missing axonal activity causes the myelenating oligodendrocytes to undergo apoptosis (Barres et al. 1993). In the central nervous system (CNS), the cell debris is then cleared by microglia, which enhances the formation of a glia scar. In the peripheral nervous system (PNS) on the other hand, the debris of the Schwann cells, which provided the myelin sheath of the lost fiber, is cleared by macrophages. This difference in debris clearance is thought to explain the time difference in Wallerian degeneration: In the mammalian PNS the process takes 7 to 14 days, whereas it takes months to years in the CNS (Vargas and Barres 2007).
It has to be noted, that in Wallerian degeneration the distal fiber is selectively degenerated while sparing the cell's soma. This quality separates Wallerian degeneration from the neurodegenerative processes described above.

1.2 Experimental approach

Within this project two methods were evaluated for their potential to redirect neuroblast migration from the RMS towards a brain lesion. Excitotoxic lesions of the medial prefrontal cortex of adult rats served as a model for a structural brain damage. The underlying principles of the experimental approach will be introduced here.

1.2.1 Excitotoxicity and the ibotenic acid model

Under physiological conditions levels of Ca^{2+} within the vertebrate CNS are tightly regulated in order to maintain a gradient between low intracellular and a high extracellular Ca^{2+} concentration (Zündorf and Reiser 2011). This way, the interplay of ion channels, Ca^{2+} binding proteins, Ca^{2+} pumps and intracellular Ca^{2+} storage capacities allows the cation to be utilized in a variety of signaling systems. Ca^{2+} signals are not only triggered by membrane depolarization and extracellular agonists, but also due to mechanical stretch of the cell, noxious insults or by intracellular messenger systems (Zündorf and Reiser 2011). However, Ca^{2+} signaling is also involved in mechanisms leading to the degeneration of neurons.

If present at excessive levels, glutamate, the major excitatory neurotransmitter in the vertebrate brain, triggers neurotoxic processes. Overactivation of the ionotropic glutamate receptors leads to increased Na^{+} and Ca^{2+} influx through the ionotropic NMDA, AMPA, and kainate glutamate receptors and consequently an additional Ca^{2+} influx through voltage gated ion channels (Choi 1987). The increased Ca^{2+} levels lead to the release of cytochrome c from the mitochondria, which in turn triggers apoptosis (Mattson and Chan 2003). Additionally, binding of cytochrome c to InsP3 receptors at the endoplasmic reticulum, an internal buffer for cytosolic Ca^{2+}, triggers even more Ca^{2+} release into the cytoplasm (Szydlowska and Tymianski 2010). Furthermore, ATP production in the mitochondria is impaired by the increased Ca^{2+} levels, whereas enzymes that produce reactive oxygen species are activated (Szydlowska and Tymianski 2010) - two mechanisms that further contribute to neurotoxicity.

This so called excitotoxicity is involved in multiple known neurodegenerative processes – either directly due to increased glutamate levels, for example in ischemia (Bano and Nicotera 2007), stroke (Lo et al. 2005), amyotrophic lateral sclerosis (Spreux-Varoquaux et al. 2002), seizure induced lesions (Haglid et al. 1994),or after TBI (Yi and Hazell 2006), or indirect, due to a Ca^{2+} dysregulation, which causes the cells to be more susceptible to excitotoxicity. The last mentioned phenomenon is thought to contribute to the degenerative processes in aging (Foster 2007) and Alzheimer’s disease (Wang et al. 2017).

Apart from glutamate, other receptor agonists can trigger excitotoxicity analogously. The ibotenic acid used in the studies presented here is an agonist both for metabotropic

glutamate receptors, as well as the ionotropic NMDA receptor. However, the excitotoxic properties are mediated through the last mentioned receptor type (Zinkand et al. 1992). As a model for neurodegenerative processes ibotenic acid provides a high validity. In contrast to lesioning methods such as aspiration or thermocoagulation, it triggers a process directly involved in known neurodegenerative processes (see above) or at least activates the downstream mechanisms of apoptosis and/or necrosis, involved in probably any pathological brain damage. Ibotenic acid injections also allow for more spatially confined lesions than cerebral artery occlusions (Chiang et al. 2011) or other excitotoxic agents such as kainate (Jarrard 1989) (for a discussion on the spatial selectivity achieved in the present project see 4.1.3).

Taken together, ibotenic acid provides a valuable model for a broad spectrum of neuronal cell loss. However, other methods might be more appropriate if a specific method of brain damage should be modeled.

1.2.2 The medial prefrontal cortex in rats

The medial prefrontal cortex (mPFC) in rats is located along the medial wall of the forebrain. It consists of a dorsal part, which includes the anterior cingulate and the medial precentral cortices, and a ventral subdivision, which consists of the infralimbic and prelimbic cortices (Uylings et al. 2003; Heidbreder and Groenewegen 2003).

Various studies on lesions of the mPFC provide insight into the function of this structure. Rats with a lesioned mPFC show deficits in working memory tasks such as delayed response (Kolb et al. 1974) or delayed alternation (Divac 1971; Wikmark et al. 1973; Deacon and Rawlins 2006). Also attention (Muir et al. 1996) and attentional set shift (Birrell and Brown 2000) are impaired by lesions of the mPFC. Furthermore, the mPFC is involved in fear and anxiety (Lacroix et al. 2000), as well as the regulation of emotion (Quirk and Beer 2006).

The main reason why this project opted for lesions of the mPFC was based on the anatomical position of this structure: The RMS runs through the forebrain just about a millimeter ventral and only slightly lateral to the infralimbic cortex (cp. fig 2.1 b). In the context of the first study this allows the tract of laminin to be positioned between the RMS and the center of the lesion by means of a relatively simple stereotactic surgery, without the need for tilted angles of approach. Furthermore, the length of the injected

laminin tract of 1.7 mm seemed feasible, but far enough to differentiate laminin-induced migration from a hypothetical spontaneous migration of neuroblasts. In the second study neuroblasts were required to migrate towards the lesion without artificial guidance. Again, a lesion directly at the RMS would not have gained insight into such chemoattraction, since the dispersal of neuroblasts from the RMS is a known effect of endoN treatment (Ono et al. 1994), whereas migratory cues released too far away from the RMS might not be sufficient for guidance of the neuroblasts.

Furthermore, the effects of a hypothetical reinnervation of the lesioned mPFC on the behavioral deficits described above could be experimentally evaluated with respect to the preexisting literature on this topic.

1.2.3 Adult neurogenesis as a potential resource for brain repair

The approaches presented in this thesis are both based on the utilization of physiological neurogenesis. Two distinct zones within the adult mammalian brain, the subgranular zone of the dentate gyrus and the subventricular zone (SVZ), constantly generate new neuronal precursor cells.

Within these tissues a subpopulation of astrocyte-like cells, the type-B stem cells, can undergo mitosis to either duplicate or divide asymmetrically, generating type-C progenitor cells. These cells in turn are capable of undergoing asymmetrical mitosis, resulting in cells with limited proliferative potential, the type-A progenitor cells or so called neuroblasts. These cells are likely only capable of differentiating to a neuronal or glial cell type (Doetsch et al. 1997; Gage 2000; Ming and Song 2011).

The SVZ-derived neuroblasts migrate through a predesignated route, the rostral migratory stream (RMS), through the forebrain towards the olfactory bulb (OB). The RMS is a tube, formed by a scaffold of astrocytes and blood vessels (Whitman et al. 2009) within which the neuroblasts travel in a process called chain migration: The cells use each other as a substrate to migrate, independently of the neighboring astrocytes (Wichterle et al. 1997). However, these astrocytes are not only thought to provide a physical boundary, hindering the neuroblasts from leaving their path, but also provide chemical directional cues (Ghashghaei et al. 2007). Once the neuroblasts reach their destination, the OB, they migrate radially and differentiate into granule and periglomerular neurons (Mouret et al. 2009). Remarkably, only half of the cells that

reach the OB survive for more than a month (Petreanu and Alvarez-Buylla 2002). This process of elimination of new cells rather than just replacing the turned over ones seems to be essential for optimization of olfaction in rodents (Mouret et al. 2009).

1.2.4 Regulation of neuroblast migration

Cell migration is crucial in the development of the CNS. Young neurons, generated in the ventricular and subventricular zone of the developing brain have to migrate towards their final destination (Lois and Alvarez-Buylla 1994; Hatten 1999). But also the neuroblasts generated by the adult SVZ have to migrate a remarkable distance through the forebrain towards the OB. Here, two modes of migration are distinguished: During radial migration, the neuroblasts follow guidance cues on the surface of radial glia cells, which provide a scaffold for the migrating cells (Marín and Rubenstein 2003). Tangential migration, in contrast, describes the glia-independent pathway (O'Rourke et al. 1995). While radial migration is predominantly involved in short distance relocation, tangential migration enables cells to follow complex pre-determined paths throughout the developing brain (Marín 2013).

In the case of the SVZ-derived neuroblasts first tangential migration through the RMS is observed, followed by radial migration within the OB. Although the underlying mechanisms of these processes are not fully elucidated yet, a number of regulators for the adult neuroblast migration have been identified. First, the formation of migratory chains of RMS-neuroblasts is affected by several extracellular matrix proteins. Laminin is not only a direct chemoattractant for neuroblasts (Emsley and Hagg 2003), it also promotes chain formation and plays a role in maintenance of the glia tube, surrounding the RMS (Belvindrah et al. 2007). Also the cell surface receptors ApoER2 and VLDLR, likely activated by F-Spondin, a protein involved in axonal pathfinding (Burstyn-Cohen et al. 1999), are a prerequisite for chain formation and the migration of neuroblast from the SVZ into the RMS (Andrade et al. 2007).

Furthermore, certain tyrosine kinase receptors are involved in migration and proliferation of neuronal precursor cells. Blockage of the Eph tyrosine kinase receptors from their ligands ephrins-B2/3 leads to disruption of neuroblast migration and an increase in proliferation within the SVZ (Conover et al. 2000). Depending on the present ligand, another tyrosine kinase receptor, the ErbB4 receptor, is involved in the regulation of cell

aggregation (induced by NRG1) or progenitor cell proliferation (NRG2) (Ghashghaei et al. 2006).

The migration of neuroblasts is directed by chemotactic cues. The proteins Slit1 and 2 and the corresponding receptors Robo2 and Robo3 have been demonstrated to mediate repulsion of immature neurons (Wu et al. 1999; Ward et al. 2003). Slits are expressed in the lateral septum, the SVZ and the RMS, directing neuroblasts to migrate caudally and probably also hindering the cells from leaving the RMS (Nguyen-Ba-Charvet 2004). Several chemokines have been demonstrated to act chemoattractant on neuroblasts. For example, VEGF, SCF, SDF-1α and MCP-1 each trigger neuroblast migration *in vitro* (Xu et al. 2007). Furthermore, Prokineticin 2 not only acts as a chemoattractant *in vitro* (Prosser et al. 2007), also the expression patterns (Cheng et al. 2006) and results from knock-out studies (Ng et al. 2005) indicate an involvement in neuroblast migration *in vivo*. Moreover, the neurotrophic factors GDNF and BDNF have been found to induce cell motility and act as a chemoattractant on neuroblasts (Paratcha et al. 2006; Chiaramello et al. 2007).

Once the chains of neuroblasts have reached the olfactory bulb, the cells detach from each other and switch from the tangential migration mode towards radial migration. One of the initiators of this detachment is the glycoprotein Reelin, which is highly expressed in the OB (Ramos-Moreno et al. 2006). Reelin has been found to trigger detachment of neuroblasts from each other *in vitro*, and neuroblasts in mice, lacking Reelin accumulate at the ventral end of the RMS, unable to enter radial migration (Hack et al. 2002). Similarly, lack of the glycoprotein Tenascin-R reduces radial migration in the adult OB (David et al. 2013).

Little is known about the translation of receptor signals to changes in the cytoskeleton, necessary to initiate actual migration in neuroblasts of the adult brain. However, similar to the process of axonal path finding, the GTPases of the Rho family seem to be key mediators (Park et al. 2002). Once activated, these enzymes trigger actin polymerization, which in turn leads to a directed change of cell morphology (Luo 2000; Sit and Manser 2011). Furthermore, DCX, a microtubule-associated protein which is used as a marker for neuroblasts in this project, is crucial for the microtubule dynamics during cell migration. Physiologically, the bipolar migrating cell first extends the leading process towards the orientation of migration. Then the centrosome is located in the lead-

ing process, followed by a translocation of the cell's nucleus (Tsai and Gleeson 2005). This last step cannot be executed in transgenic mice, lacking DCX, which leads to a marked decrease of migration speed and a multipolar morphology of the neuroblasts (Koizumi et al.).

1.2.5 Hypotheses

The idea that the SVZ-derived neuroblasts might be utilized to repair structurally damaged brain tissue has been postulated before (Gage 2002; Emsley et al. 2005; Battista and Rutishauser 2010; Bonfanti 2013), but so far no effort has been made to actively redirect the cells from their predesignated path to an actual brain lesion. However, several previous findings suggest that brain repair based on endogenous neuroblasts might be feasible.

First, spontaneous migration of neuroblasts to lesions in the vicinity of the SVZ has been described in other studies (Jin et al. 2003; Lee 2006; Kunze et al. 2015). Therefore, it seems likely that migrating neuroblasts can be guided by substances released from cells that recently died, either pathologically or due to the physiological turn over in the OB. Furthermore, implantation studies on grafted neuroblasts indicate that donor cells from embryonic (Shin et al. 2000) and adult (Gage et al. 1995) animals differentiate and integrate according to the surrounding tissue. Taken together, it is plausible that adult generated SVZ neuroblasts are generally capable of performing structural brain repair but hindered by the rigidity of their migration path through the RMS.

In the scope of this thesis two methods were applied in order to overcome this limitation: the glycoprotein laminin has been identified as a key migratory cue for SVZ-neuroblasts in a previous study (Emsley and Hagg 2003). The same publication also demonstrated that an injection of laminin close to the RMS is capable of diverting the cells from their migration route and disperse into the surrounding tissue. The application of a tract of laminin, reaching from the RMS towards a brain lesion, should not only allow neuroblasts to leave the RMS, but also serve as a guide towards the affected tissue.

The other approach is based on the suppression of neuroblast chain formation in the RMS. The enzyme endoneuraminidase-N (endoN) has been used before (Ono et al. 1994; Hu et al. 1996; Chazal et al. 2000) to achieve this by specifically removing the polysialic acid (PSA) moiety from the neuroblasts. As a consequence the migrating cells

in the RMS migrated slower and did not form the typical longitudinal aggregates. Furthermore, the individual neuroblasts were able to leave the RMS. Importantly, the neuroblasts' ability to migrate radially within the OB remained largely unchanged (Ono et al. 1994).

From all findings presented above, the following fundamental hypotheses have been developed and will be evaluated in this thesis.

(1) Both the injection of laminin in the vicinity of the RMS and the intrventricular injection of endoN cause neuroblasts to leave the RMS.

In principle, this has been demonstrated before. However, all the key studies cited above have been conducted on mice, not rats and with slightly different techniques than used here.

(2) The neuroblasts migrate towards the lesion site spontaneously.

It is a prerequisite for successful brain repair that new cells are located where they are supposed to replace lost cells. For the laminin study the neuroblasts are expected to follow the injected laminin tract from the RMS towards the brain lesion and then migrate within the damaged tissue. The endoN-treatment only allows neuroblasts to leave the RMS – their migration towards the brain lesion is required to occur unassisted.

(3) The neuroblasts differentiate into neurons and integrate into the surrounding neuronal tissue.

In the project presented here, no additional steps to influence the fate of relocated neuroblasts have been taken. However, the results of transplantation studies give reason to believe that the microenvironment within the lesioned target tissue influences the differentiation of neuroblasts into a functionally integrated neuron.

2 Redirection of neuroblast migration from the rostral migratory stream into a lesion in the prefrontal cortex of adult rats[1]

Jannis Gundelach*, Michael Koch
Department of Neuropharmacology, Center for Cognitive Sciences, University of Bremen, PO Box 330440, 28334 Bremen, Germany

***Corresponding Author:**
Jannis Gundelach
Mail address: Department of Neuropharmacology, Center for Cognitive Sciences, University of Bremen, PO Box 330440, 28334 Bremen, Germany
Email: J.Gundelach@uni-bremen.de

2.1 Abstract

Clinical treatment of structural brain damage today is largely limited to symptomatic approaches and the avoidance of secondary injury. However, neuronal precursor cells are constantly produced within specified regions of the mammalian brain throughout life. Here we evaluate the potential of the known chemoattractive properties of the glycoprotein laminin on neuroblasts in order to relocate the cells into damaged brain areas. Injection of a thin laminin tract, leading from the rostral migratory stream (RMS) to an excitotoxic lesion within the medial prefrontal cortex of rats, enabled neuroblasts to migrate away from their physiological route towards the olfactory bulb into the lesion site. Once they reached the damaged tissue, they migrated further in a non-uniform orientation within the lesion. Furthermore, our data indicate that the process of diverted migration is still active six weeks after the treatment and that at least some of the neuroblasts are capable of maturing into adult neurons.

Keywords: Neuroblast migration; Rostral migratory stream; Brain lesion; Laminin; Migration cue; Structural recovery

2.2 Introduction

The human brain is at constant danger of sustaining structural damage, either due to trauma or neurodegenerative diseases. Although the functional consequences of brain lesions can be somewhat compensated (Nithianantharajah and Hannan 2011), and some symptoms can be treated pharmacologically (Bartus 2000; Connolly and Lang 2014), so far no cure for structural brain damage exists.
However, it is known that the adult mammalian brain produces new neuronal precursor cells in distinct brain areas, the dentate gyrus of the hippocampus (Altman and Das

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1965; Gage et al. 1995) and the subventricular zone (SVZ) of the lateral ventricle (Altman 1969; Reynolds and Weiss 1992). In these regions neural progenitor cells give rise to a special type of neuronal precursor cells, the so called neuroblasts. These cells are capable of undergoing mitosis but show a limited proliferative potential, since these cells only differentiate to neurons under physiological conditions (Jablonska et al. 2010).

While neuroblasts generated in the adult dentate gyrus do not migrate to targets outside the hippocampal formation, those that originate from the SVZ travel through the forebrain towards the olfactory bulb where they differentiate into granule and periglomerular cells (Mouret et al. 2009). Therefore, high numbers of these neuroblasts continuously move through the rostral migratory stream (RMS) (first described in (Altman 1969)). This path of migration is densely surrounded by astrocytes, which form a glial tube that prevents the migrating cells from dispersing into surrounding tissue (Lois et al. 1996; Peretto et al. 1997). The SVZ-derived cells form longitudinal aggregates and migrate along the RMS independently of the guidance of radial glia or axonal processes (Wichterle et al. 1997). During this so called chain migration the cells interact with each other and migrate at a relatively high speed of more than 70 µm/hr (Nam et al. 2007). However, this process is not uniform, since cells may stop migrating or even move caudally through the RMS (Nam et al. 2007).

The regulatory processes involved in the generation and guidance of SVZ-derived neuronal progenitor cells are complex and not yet fully understood. While the number of newly generated neuroblasts entering the RMS depends on connectivity (Jankovski et al. 1998) and activity of the olfactory bulb (OB) (Pothayee et al. 2017), the general process of cell migration in the RMS seems to be OB independent (Jankovski et al. 1998; Kirschenbaum et al. 1999). The direction of migration is rather controlled locally by the microenvironment within the migratory path, which in turn is possibly controlled by the ensheathing astrocytes of the RMS (García-Marqués et al. 2010). A number of chemoattractive and chemorepulsive substances, such as various neurotrophic factors and extracellular matrix proteins, have been identified so far (for reviews see (Sun et al. 2010) and (Leong and Turnley 2011)).

An earlier study by Emsley and Hagg (2003) described the role of the interaction between laminin and its receptor integrin on neuroblast migration. Selective antibody blockade revealed that both the α6 and β1 subunit of integrin are required for RMS migration. Additionally, an antibody against the β1 subunit led to a disruption of the typical neuroblast chains and a dispersal of cells from their migration path into the surrounding tissue.

Furthermore, the authors demonstrated that the chemoattractive properties of laminin, or a shorter peptide, representing the α6β1-integrin binding site of laminin, can be utilized to redirect neuroblasts from the RMS into the surrounding tissue. Injection of this laminin subunit dorsal to the RMS led to an accumulation of cells, which are positive for the cell proliferation marker BrdU as well as PSA-NCAM and Tuj1, two markers for immature neurons. Furthermore, the authors injected laminin dorsally to the RMS, while

retracting the injection cannula, resulting in a narrow, 2 mm long tract of the protein. Seven days later they found cells positive to the above mentioned markers at the injection tract and dorsal to it, demonstrating that migrating neuroblasts were diverted from the RMS into the injected tract of laminin.
In the present study, we apply this method in a brain lesion model and test the neuroblasts' ability to a) migrate to the lesion along the injected tract of laminin, b) disperse from the injection tract into the lesioned tissue, and c) assess the number of neuroblasts within the lesion. Furthermore, we opted for a relatively long survival time after the laminin application in order to evaluate the long-term effects of the treatment in terms of survival of the cells, possible differentiation, and a sustained flow of neuroblasts into the lesion site. Overall, this study was designed as a proof-of-concept of redirection of neuroblasts into a lesion as a possible future treatment of structural brain damage that only requires a single, relatively simple, minimal invasive surgery.

2.3 Material and methods

2.3.1 Subjects

A total of 25 adult (age 3.5 to 6.8 months) male Wistar rats (Charles River, Germany) were used in this study. The animals were kept under standard housing conditions (12 h light/dark cycle, lights on at 7 a.m., water *ad libitum*, standard lab chow 12 g/rat/day) in groups of 4 to 6 animals per cage. The experiments were performed in accordance with the National Institutes of Health ethical guidelines for the care and use of laboratory animals for experiments and were approved by the local animal care committee (Senatorische Behörde, Bremen, Germany).

2.3.2 Treatment

All animals underwent two stereotactic microinjections. First they were lesioned bilaterally by injection of ibotenic acid (Cayman Chemical Company, MI, USA; 6.7 mg/ml saline; injection volume of 0.4 µl at 0.3 µl/min infusion rate) into the medial prefrontal cortex (3.2 mm rostral, ±0.5 mm lateral, -4.4 mm ventral, all relative to bregma).
Five days later the laminin tract (L2020, Sigma-Aldrich Chemie GmbH, Germany) was applied bilaterally. For this purpose the injection cannula (custom made from 30 G epidermal stainless steel cannula) was placed directly dorsal and slightly medial to the RMS (rc. +3.0 mm; l ±1 mm; vd. -6.3 mm, relative to bregma; see Fig 2.1). After a dwell time of three minutes the laminin solution (0.25 µg/ml; Lam group) or vehicle (phosphate buffered saline, PBS; C group) was injected at a constant flow rate (0.16 µl/min) by means of an injection pump while the injection cannula was slowly retracted dorsally (0.6 mm/min) into the lesion site (vd. -4.6 mm). The injection volume of 0.48 µl was chosen to match the volume of the resulting tract. After another three minutes the cannula was fully retracted before the trepanations were closed with bone wax (SMI AG, Belgium). After application of an antiseptic ointment (Betaisodona, Mundipharma, Germany) and a local anaesthetic (Xylocain 2 %, AstraZeneca GmbH,

Germany) the skin was sutured. An additional group was treated with a higher concentration of laminin (0.25 µg/µl; LamHi group).
All surgical procedures were performed under isoflurane (CP-Pharma, Germany) anaesthesia (vaporizer in circle system). The spontaneous breathing rate (BR) was constantly monitored and the vaporizer setting was adjusted to keep BR at 40 to 60 breaths per minute.

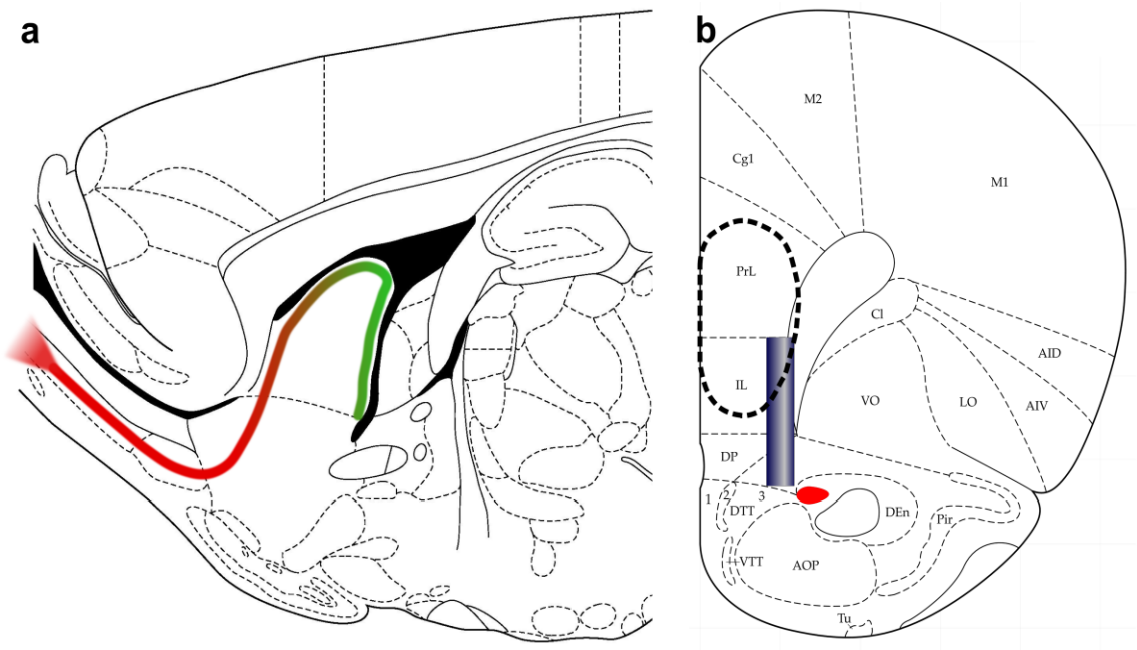

Fig 2.1: a) Sagittal view of the physiological path of neuroblasts in adult rats: Neuroblasts are generated at the walls of the lateral ventricle (green) and migrate along the RMS (red) through the forebrain towards the OB where they disperse and differentiate (position of the RMS illustrated according to (Altman 1969)). **b) Experimental procedure:** Laminin was injected while retracting the cannula dorsally, resulting in a narrow tract of laminin (blue) between the RMS (red) and the excitotoxic lesion (-6.3 mm to -4.6 mm ventral to bregma). The approximate position of lesion is represented by the dashed line. The images show the sagittal section 1.4 mm lateral to bregma (a) and the coronal brain section 3.2 mm rostral to bregma (b) (modified from (Paxinos and Watson 1998))

2.3.3 Immunhistochemistry

After a survival time of 6 weeks after the second intracranial injection, the animals were sacrificed by intraperitoneal injection of sodium pentobarbital (200 mg/kg bodyweight at a concentration of 20 mg/ml PBS; Sigma-Aldrich Chemie GmbH, Germany), before being transcardially perfused with PBS followed by 4 % paraformaldehyde solution. Immunhistochemistry was performed on 40 µm thick coronal cryosections (distance of 240 µm). Sections were blocked free floating in a 10 % normal donkey serum (Jackson ImmunoResearch, PA, USA) in PBS and 0.05 % Triton-X. The same solution was used for incubation with NeuN (1:1000; Millipore Rabbit anti-NeuN (RRID: AB_10807945))

and doublecortin (DCX) (1:1000; Santa Cruz Doublecortin Antibody (C-18) (RRID: AB_2088494)) antibodies for 72 h at 4 °C. After blocking in 10 % bovine serum albumin (Sigma-Aldrich Chemie GmbH, Germany) for 1 h, the sections were incubated in secondary antibody solution (CruzFluor 488 Donkey anti-Rabbit IgG (RRID: AB_10989100), 1:2000 and Santa Cruz Biotinylated Donkey anti-Goat (RRID: AB_631726), 1:1000) for 48 h at 4 °C. The streptavidin-conjugated fluorescent dye (Jackson Streptavidin-Alexa Fluor 568 (RRID: AB_2337250), 1:2000) was allowed to incubate for another 24 h at room temperature. Subsequently, the sections were counterstained in 0.9 w/v % Sudan Black (Acros Organics, Belgium) in 70 % ethanol and then mounted, air dried, and coverslipped. Additionally, exemplary brain slices were stained against DCX and the astrocyte marker glial fibrillary acidic protein (GFAP) in order to reassure the specificity of the DCX antibody for neuroblasts. The staining procedure followed the same protocol described above, but the primary antibody for NeuN was replaced by Dako Rabbit anti-GFAP (RRID: AB_10013382) at the same concentration.

2.3.4 Image acquisition and analysis

Images were acquired on a fluorescent microscope (Axioscope 100, Carl Zeiss AG, Germany) and a monochrome digital camera (Spot, Visitron Systems GmbH, Germany). Brains with severe misplacement of the laminin tract or the lesion were excluded from the analysis, resulting in group sizes of 12 hemispheres for the sham Laminin injected (C) group, 19 for the laminin injected (Lam) and 8 for the high dose laminin (LamHi) group. Overview images were taken at the rostro-caudal position of the laminin tract and later stitched in Microsoft Image composition editor (Microsoft Cooperation, version 1.4.4.0) using the planar motion 1 (rigid scale) setting. Adjustment of brightness and contrast as well as creation of scale bars were done in the ImageJ (version 1.49h) (Schneider et al. 2012; Schindelin et al. 2015) based software FIJI (Schindelin et al. 2012).

The brain lesions were identified in the NeuN staining whereas the RMS showed strong immunoreactivity against DCX. The laminin/vehicle injection tract was clearly visible in both the NeuN and DCX stainings, either as a fissure in the slice or due to strong background staining at the site of the injection (see Fig 2.3). The hemispheres of the animals were analyzed separately. Sections with insufficient staining or severe damages at the area of interest were excluded from the analysis. In the section closest to the laminin tract or the corresponding vehicle injection the lesion was then surrounded with the polygon tool in FIJI to measure its size. Within this region DCX positive cells were counted. Only cells with a luminance similar to that of the RMS and with the typical morphology of neuroblasts were counted (see Fig 2.2). This analysis was performed by an observer blind to the treatment. To account for the thickness of the brain slices, the number of cells was assessed per region of interest volume (i.e. area of interest multiplied by the slice thickness). The statistical analyses were conducted in IBM SPSS Statistics (version 20 for Windows).

2.4 Results

2.4.1 Ibotenic acid induced brain lesion

Microinjections of ibotenic acid reliably caused brain lesions, characterized by the absence of NeuN positive cell bodies. The lesion sites showed some variability in size and shape but were mostly limited to the prelimbic and infralimbic cortex (see Fig 2.1 for the approximate position). In some cases parts of the cingulate cortex, area 1 and the dorsal peduncular cortex were affected. At the coronal plane of the laminin injection (3.0 mm rostral to bregma) the lesions stretched over an area of 1.24 mm^2 ±0.15 mm^2 (standard error of the mean; n = 37 hemispheres).

2.4.2 Two different populations of cells are positive for DCX

Two different populations of cells were identified in the lesions: One type of cells showed a distinctly weaker fluorescence and astrocyte-like morphology with multiple processes (*stellate cells*; cf. (Kunze et al. 2015)). The other cell type showed only one or two processes originating from a smaller soma and a much stronger staining (*polar cells*). In exemplary brain slices we reproduced the finding that the DCX-immunoreactive *stellate cells* were also positive for the astrocytic marker GFAP, but none of the *polar cells* were (Fig 2.2). Furthermore, the *stellate* cell type was only found within the lesions of laminin treated animals, whereas the *polar cell* type was also observed in the SVZ, the RMS, and the olfactory bulb. Moreover, these cells were found between the RMS and the lesion in laminin treated animals. Overall, the distribution and morphology of GFAP positive cells within the lesion corresponded to that described earlier for excitotoxic lesions (Dusart et al. 1991), with the addition that a subpopulation of these cells were also immunoreactive for DCX. For the quantification of neuroblasts only cells that were clearly characterized as *polar* type were counted.

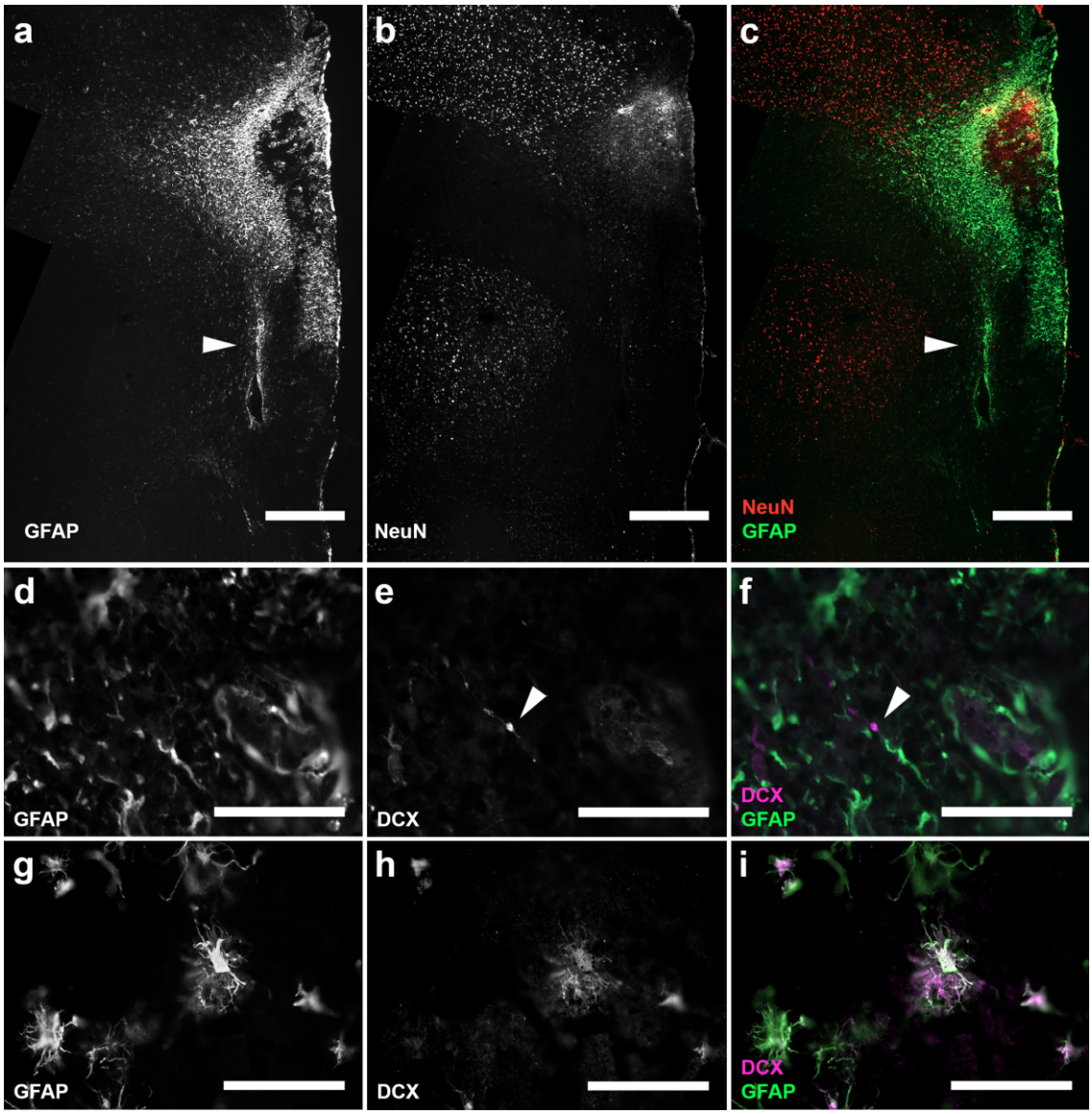

Fig 2.2: a - c: Low magnification photomicrographs of the gliotic response six weeks after the excitotoxic lesion, coronal section, 3.0 mm rostral to bregma. GFAP-positive cells have formed a glial scar around the lesion. A smaller number of GFAP-positive cells are also found around the laminin tract (arrowheads in a and c; scale bars: 500 µm). **d - i: Two different cell populations found in brain lesions are immunoreactive for DCX. d - f: Example of a polar neuroblast immunoreactive for DCX (arrowheads in e and f).** These cells show one long process extending from the soma towards the migration direction (leading process) and often another, shorter process oriented towards the opposite direction (trailing process). These cells do not show any overlap with GFAP staining (d and f). **g - i: Example of a stellate cell positive for DCX (h).** The stellate cells show a round morphology with a comparatively large soma and multiple processes in all directions. They are also positive for the astrocyte marker GFAP (g), although only a small portion of GFAP-positive cells are also positive for DCX (cp. overlay in i). Note that here contrast and brightness are adjusted for maximum visibility of the cells. Side by side the polar neuroblasts show markedly brighter fluorescence than the stellate cells in the DCX assays. Scale bars in d - i: 100 µm

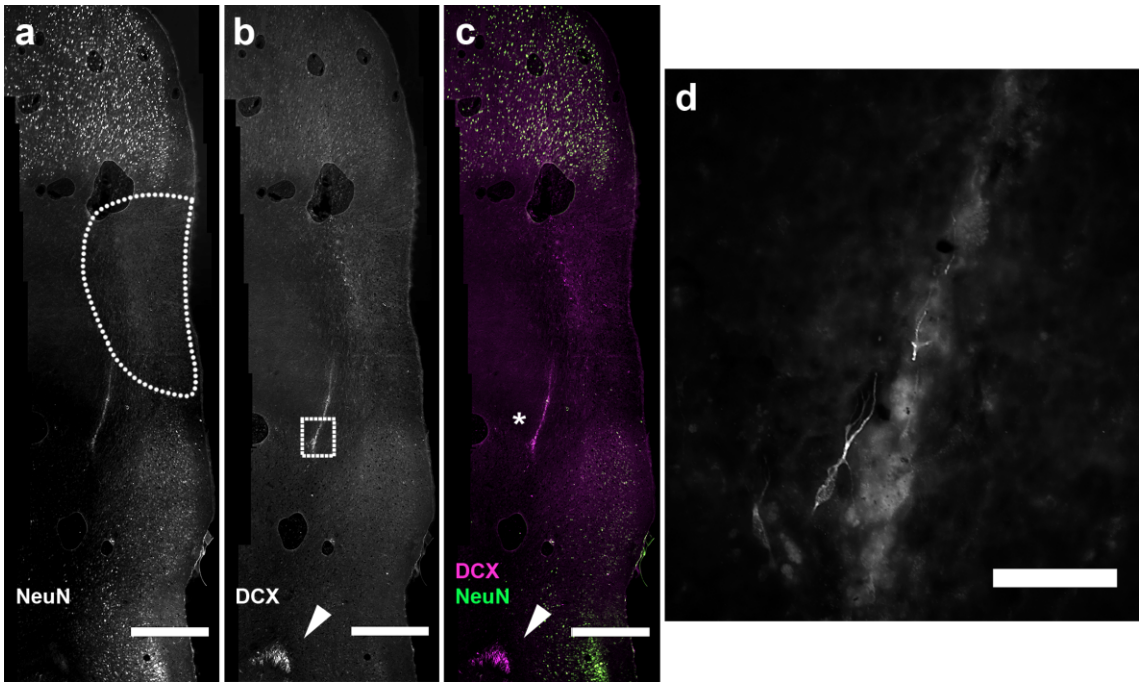

Fig 2.3: DCX-positive neuroblasts following the laminin-tract dorsally towards the lesion site. a – c: Low magnification of coronal section showing the lesion (circumscribed by dotted line in a) and the RMS (arrowheads in b and c). The laminin tract is visible by the line of stronger background staining in vertical direction (marked with an asterisk in c). Scale bars: 500 µm. **d: Detail of the DCX staining (position marked by dashed box in b).** The leading processes of the neuroblasts are oriented parallel to the path of the laminin injection.and directed dorsally, towards the lesion. Scale bar: 50 µm

2.4.3 Neuroblasts follow the laminin tract, and then disperse into the lesion site in a non-uniform orientation

Neuroblasts were found in 73.7 % of the lesions of the Lam group (n = 19 hemispheres), whereas all of the hemispheres of the LamHi group (8 hemispheres) showed DCX-positive cells within the lesion site. Two of the twelve control hemispheres (16.7 %) had DCX-positive cells in the lesion site.

All neuroblasts found between the lesion site and the RMS were located in close vicinity to or directly at the laminin tract with their processes oriented in the direction of the injection path (Fig 2.3). In contrast to the physiological migration through the RMS these cells did not show any signs of chain formation. The RMS of laminin treated animals did not show any visible alterations from that of the control animals, indicating that the physiological SVZ-OB migration was largely unaffected.

Neuroblasts in the lesion were not restricted to the laminin tract and did not show a coherent orientation (Fig 2.4 a – c). Furthermore, many of these cells showed the canonical morphology of orientation changing neuroblasts (Martinez-Molina et al. 2011). This means that the cells kept migrating within the lesion probably following different orientation cues. In six hemispheres of the laminin treated groups single cells, both stained

for DCX and NeuN were observed (Fig 2.4 i – k). These cells were all located at the border of the lesions and most of them resembled the morphology of adult neurons with long processes towards the intact surrounding tissue.

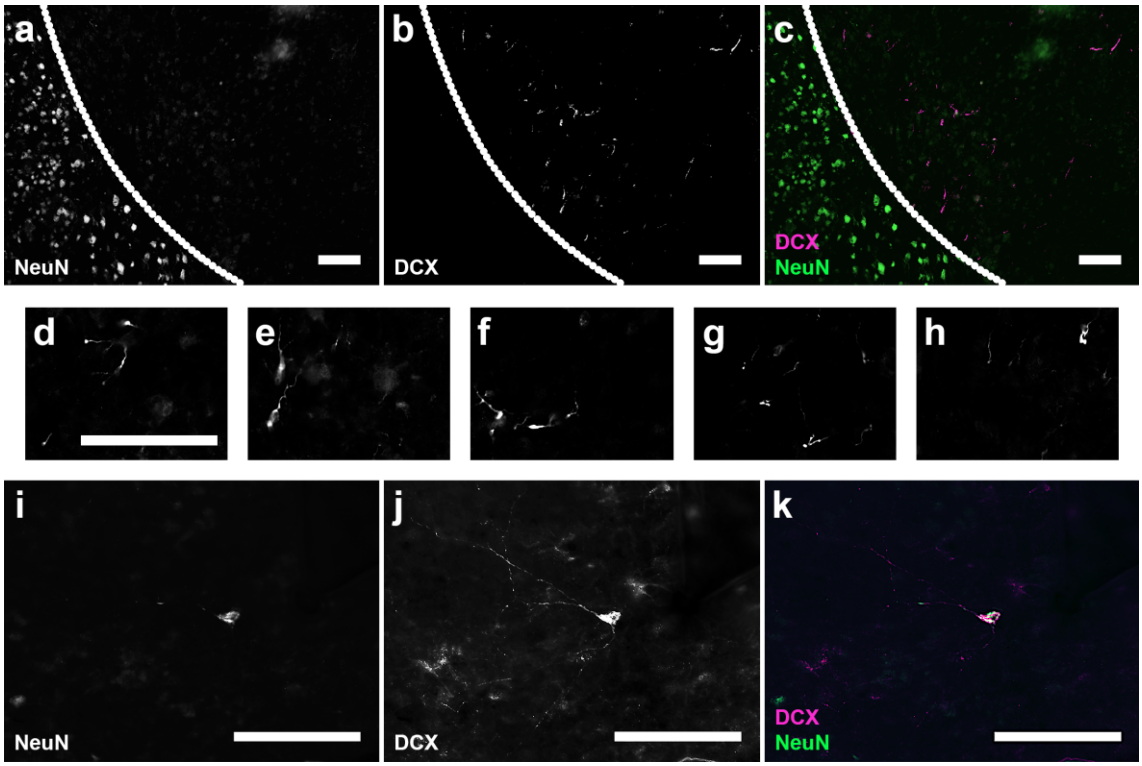

Fig 2.4: a – c: Neuroblasts within the lesion; dotted line represents the border of the lesion. After following the laminin-guided migration from the RMS the neuroblasts (DCX in b) disperse within the lesion site (area without NeuN-positive cells in a). They no longer show a uniform orientation and many of the cells resemble typical direction changing neuroblasts (Martinez-Molina et al. 2011). **d – h show higher magnifications of neuroblasts depicted in b (magnification is identical for d – h). i – k: Example of a cell within the confines of the lesion, positive for the neuroblast marker DCX (j) and the marker for adult neurons, NeuN (i).** The cell shows long processes towards the surrounding intact neuronal tissue. All scale bars: 100 µm

2.4.4 Laminin dose affects the reliability of the method but not the density of relocated cells

DCX-positive neuroblasts were counted within the lesion site at the coronal plane of the laminin or vehicle injection (Fig 2.5). A Kruskal-Wallis H test showed a significant difference in cell density between the treatment groups ($X^2 = 16.29$, $p < 0.001$, mean ranks: C: 9.75; Lam: 22.89; LamHi: 28.50). The pairwise comparison demonstrated a significant difference between the mean cell density of the C group and both the Lam ($p = 0.003$) and LamHi ($p < 0.001$) group, but not between the Lam and LamHi group ($p = 0.687$). However the higher dose of laminin caused a more reliable relocation of

neuroblasts towards the lesion, which resulted in a lower variability within the LamHi group (interquartile range (IQR): 0.61 to 20.86 cells/mm³) compared to that of the Lam group (IQR: 13.54 to 18.60 cells/mm³).

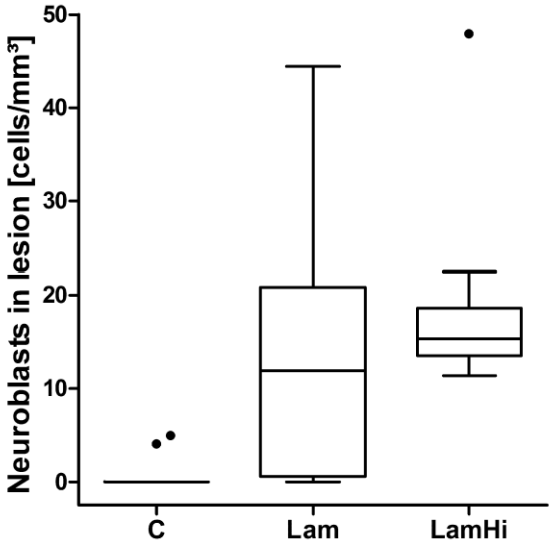

Fig 2.5: Neuroblasts within the brain lesion site presented as cells per mm³ of lesioned tissue. Both treatments led to a significantly higher density of neuroblasts than the vehicle application (Kruskal-Wallis H test, $X^2 = 16.29$, $p < 0.001$, mean ranks: C: 9.75; Lam: 22.89; LamHi: 28.50). According to the pairwise comparison the mean cell density differed significantly between the C group and both the Lam ($p = 0.003$) and LamHi ($p < 0.001$) group, but not between the Lam and LamHi group ($p = 0.687$). However the higher dose of laminin caused a relocation of neuroblasts towards the lesion more reliably, which resulted in a lower variability within the LamHi group (IQR: 20.25 cells/mm³) compared to that of the Lam group (IQR: 5.06 cells/mm³). Boxes represent IQR; whiskers mark highest and lowest numbers within 1.5* IQR; dots represent extreme outliers

2.5 Discussion

In the present study we demonstrate that the previously described chemoattractive properties of laminin can be utilized to redirect the migration of neuroblasts into lesions in rat brains. Our data suggest that a part of the cells travelling through the forebrain keep migrating along the surgically introduced migration path for as long as at least six weeks without disrupting the physiological migration into the olfactory bulb of the animals. Furthermore, the neuroblasts leave the laminin tract once they reach the lesion and disperse within the damaged tissue, where at least a part of them seems to mature into adult neurons.

2.5.1 Astrocytes in the lesions of laminin treated animals can be immunoreactive for DCX

Physiologically, DCX, a marker for migrating neuronal precursors, is scarcely observed outside of the neurogenic zones of the brain of rats (Omori et al. 1998; Brown et al. 2003) and humans (Verwer et al. 2007). Nonetheless, there have been reports of astrocytes, expressing DCX under pathological conditions (Verwer et al. 2007; Kunze et al. 2015). The reason for the occurrence of this microtubule associated protein in glia cells is not yet fully understood. Possible explanations include the involvement of DCX in the migration of astrocytes, but also the transdifferentiation of astrocytes towards a stem cell-like phenotype (Kunze et al. 2015). Furthermore, neuroblasts can differentiate into GFAP-positive astrocytes *in vitro* (Reynolds and Weiss 1992), so that the coexpression of GFAP and DCX for some time is conceivable. Although our data does not provide a sufficient explanation to this phenomenon, it is noteworthy that we only observed DCX-positive cells with astrocyte-like morphology and immunoreactivity for GFAP in the lesions of laminin treated animals where also DCX-positive polar neuroblasts were found. This finding goes along with the results of (Kunze et al. 2015), who identified DCX-positive astrocytes alongside DCX-positive neuroblasts that probably migrated through the striatum towards a cortical lesion. Therefore, we favor the hypothesis that DCX expression in astrocytes is related to an interaction between migrating neuronal precursor cells rather than a spontaneous reaction of astrocytes to brain damage or degeneration.
This observation suggests that if astrocytes are indeed capable of dedifferentiating towards a more stem cell-like phenotype (Leavitt et al. 1999; Steindler and Laywell 2003), this process might be promoted by the presence of neuroblasts. A similar conversion of astrocytes to DCX-positive neuroblasts has been demonstrated after *in situ* reprogramming of spinal cord astrocytes by introduction of the transcription factor SOX2 (Su et al. 2014).
However, although our data confirm that the immunohistochemical detection of DCX was not exclusive to neuroblasts, we consider the combined assessment of staining intensity and cell morphology as a reliable tool to identify neuroblasts in order to analyze their number and location within the lesioned tissue.

2.5.2 Neuroblasts follow migratory cues towards brain lesions

The SVZ and the RMS are considered distinct regions from which neuronal precursors rarely disperse into surrounding tissue under physiological conditions. However, there are a number of reports about precursor cells migrating towards damaged brain areas. For example migration of DCX-positive neuroblasts towards the striatum in a mouse model for stroke was observed (Lee 2006). Similarly, after focal ischemia DCX-positive cells were found to migrate into the striatum and the cortical regions adjacent to the infarct (Jin et al. 2003; Kunze et al. 2015). Following traumatic brain injury DCX- and PSA-NCAM-positive cells migrated towards a cortical lesion in the parietotemporal cortex in mice (Dixon et al. 2015). Taken together, these studies demonstrate that neuro-

blasts migrate towards brain lesions, independent of the nature of brain damage. However, the migratory potential deviating from the physiological route seems to be limited to regions in the direct vicinity of the neurogenic regions or the RMS. In the case of neuroblast migration towards lesions of the cortex, the interface between cortex and corpus callosum appears to provide an alternative path of migration (Jin et al. 2003). In our study the animals received excitotoxic lesions that did not touch the ventricle wall or the RMS. Hence, neuroblasts did only rarely travel towards the lesion site within the control group, which received a vehicle injection instead of the laminin tract (two out of eight hemispheres, both at a lower cell density than the laminin treated groups; cp. Fig 2.5).
In the original study that demonstrated the capability of laminin to divert neuroblasts from the RMS into the surrounding tissue (Emsley and Hagg 2003) the authors also reported single cases of neuroblasts entering the vehicle tracts. We support their original assumption that these migratory processes are triggered by substances released due to the injection-induced microlesion in the vicinity of the RMS, which concurs with the above mentioned more recent studies on spontaneous migration of neuroblasts towards brain lesions.

2.5.3 Temporal aspects of neuroblast relocation

In the present study we preferred to use the neuronal precursor and neuroblast marker DCX over the often used proliferation markers such as BrdU. In the context of this proof-of-concept study the advantages of this method outweigh the restrictions with respect to a limited insight of the temporal aspects of laminin-based neuroblast redirection. Besides demonstrating that neuroblasts could be diverted from the RMS into a brain lesion we could validate that the relocated cells are present in the lesion site even after a survival time of six weeks. Meanwhile, a proliferation marker only marks cells that undergo reproduction while the substance is present systemically so that cells produced either prior to or after the application can not be detected. Furthermore, the application of BrdU during formation of the glial scar would yield unclear results, since during this phase enhanced proliferation of glia cells is expected (Wanner et al. 2013).
However, the data at hand allow some interpretation of the underlying processes. First, in many cases neuroblasts were found close to the laminin tract between the RMS and the lesion site. This could be either interpreted as a sign of disrupted migration with cells stuck on their way towards the lesion or seen as an indicator for neuroblast migration towards the lesion still going on by the end of the experiment. Since we did not observe a single case of neuroblasts accumulating ventral to the damaged tissue, we favor the assumption that cell migration towards the lesion has not yet ceased by the time the animals were sacrificed.
Furthermore, cells resembling the morphology of adult neurons immunoreactive for both DCX as well as NeuN were found at the border of some lesion sites. This is especially remarkable since co-expression of these markers in developing neurons only occurs during a few days under physiological conditions (Brown et al. 2003). This finding implies that at least some of the relocated cells are capable of differentiating into adult

neurons. This hypothesis is further supported by the results of (Reynolds and Weiss 1992), who isolated and grafted cells from the adult rodent brain that were capable of generating new neurons. Although these authors used different cellular markers the results show striking parallels to the outcome of our study: After 21 days in culture a number of cells migrated away from the previously built cell spheres and differentiated into either a neuronal or astrocytic phenotype. The morphology of the migrating neuroblast, the astrocytes, as well as the differentiated neurons closely resembles our results (cp. (Reynolds and Weiss 1992), fig 3).
It is possible that more neuroblast have differentiated than deduced from our data, since neuroblasts that had completed maturation earlier might blend in with the NeuN-positive cells surrounding the lesion. However, this hypothesis needs to be evaluated in follow up experiments.
Another aspect that could be addressed by targeted use of proliferation markers is the long-term fate of newly generated neurons. Under physiological conditions, newly generated neuroblasts travel from the SVZ towards the OB within about seven days in rats (Peretto et al. 1997; Brown et al. 2003). At about 15 to 30 days after proliferation most of these cells differentiate into mature neurons (Petreanu and Alvarez-Buylla 2002), but only a part of these cells survive for a prolonged time (Mizrahi et al. 2006; Mouret et al. 2009). It remains to be determined if, and to what extent the fate of replaced neurons in brain lesions differs from the development of replaced neurons in the olfactory bulb.

2.5.4 Potential clinical significance of neuroblast relocation

One major objective of this study was to evaluate the method originally described by (Emsley and Hagg 2003) with regard to its potential for a future therapy of structural brain damage. Although our data provide further evidence that relocated neuronal precursor cells might contribute to a clinical application, a number of open questions need to be addressed.
First, the details of neuroblast migration in the human forebrain remain controversial. While neurogenesis in the hippocampus and SVZ of the adult human brain are undisputed (Curtis et al. 2011), the existence of a human RMS is topic of an ongoing debate. Initially, the lack of specific markers commonly found in the RMS of rodents and non-human primates (Kornack and Rakic 2001) has led to doubts, if neuroblast migration occurs in the human brain at all (Sanai et al. 2004). In later studies however, an RMS was identified, though it showed a different anatomy and a markedly lower number of migrating neuroblasts compared to the RMS of rodents (Curtis et al. 2007; Wang et al. 2011). It has to be noted that the data on human neuroblast migration is based on brains of older subjects, since only *post mortem* analysis is applicable. More recent studies reported the human RMS activity to decline drastically during infancy (Sanai et al. 2011; Bergmann et al. 2012) and that neuroblasts in adult humans might rather migrate towards the striatum instead of the OB (Ernst et al. 2014). However, it has to be determined if neuronal progenitor cells located within the adult human SVZ are susceptible to migratory cues. If these cells do not enter the RMS physiologically but can be stimu-

lated to migrate towards signal molecules, the application of a substance tract directly towards the SVZ might provide an advantageous alternative to the method used in this study.
Another important issue of neuroblast relocation presented here is the relatively low number of new cells found in the lesion site. Although even a lower number of neuronal precursor cells in damaged brain tissue have beneficial effects on functional recovery (Li et al. 2010; Dixon et al. 2015), the quantity of relocated neuroblasts needs to be significantly higher in order to compensate for the lost neuronal tissue. Nonetheless, our data indicates a long-term effect of the single laminin injection which might lead to a higher number of replaced neurons over time. Furthermore, the adult neurogenic niche is dynamically regulated by various systems (Ming and Song 2011) and the rate of neurogenesis is upregulated after neurological disorders such as stroke (Arvidsson et al. 2002) or seizures (Parent et al. 1997). A better insight into the underlying mechanisms and possible methods to influence these regulatory processes might increase the value of neuroblast redirection.

2.5.5 Conclusion

The method described here might eventually lead to a therapy of structural brain damage. It offers a comparatively simple and minimal-invasive approach and the limited proliferative potential of the neuroblasts increases the safety of this method over methods based on embryonic stem cells (Meyer et al. 2010). Moreover, transplantation studies provide evidence that redirected neuroblasts repopulate damaged brain tissue and integrate into the complex networks successfully (Shin et al. 2000; Gallina et al. 2010).

Compliance with ethical standards
Funding: This study was partly supported by the Tönjes-Vagt-Stiftung Bremen.
Conflict of interest: The authors declare that they have no conflict of interest.
Ethical approval: All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

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3 EndoN treatment allows neuroblasts to leave the rostral migratory stream and migrate towards a lesion within the prefrontal cortex of rats

Jannis Gundelach[1*], Michael Koch[1]
[1] Department of Neuropharmacology, Center for Cognitive Sciences, University of Bremen, PO Box 330440, 28334 Bremen, Germany

* **Corresponding author**
Jannis Gundelach
Mail address: Department of Neuropharmacology, Center for Cognitive Sciences, University of Bremen, PO Box 330440, 28334 Bremen, Germany
Phone: +49 421 218-62979
Email: J.Gundelach@uni-bremen.de

3.1 Abstract

The polysialic acid moiety modulates the binding properties of the neural cell adhesion molecule (NCAM). This plays a particular role in the migration of adult born neuroblasts from their area of origin, the subventricular zone, towards the olfactory bulb. It increases the migration speed of the cells and helps to prevent the neuroblasts from leaving their migration route, the rostral migratory stream. Here, we evaluated the potential of intraventricular application of endoneuraminidase-N (endoN), an enzyme that specifically cleaves polysialic acid from NCAM, in a rat model for structural prefrontal cortex damage. As expected, endoN caused the rostral migratory stream to become wider, with a less uniform cellular orientation. Furthermore, neuroblasts left the rostral migratory stream and migrated towards the nearby lesioned tissue. Despite the neuroblasts not being differentiated into neurons after a survival time of three weeks, this technique might provide a basis for a future endogenous stem cell-based therapy for structural brain damage.

3.2 Introduction

Although our understanding of the function of the brain and the constant threat of damage, either due to neurodegenerative diseases or trauma, constantly grows, so far no clinical treatment for structural brain damage has been developed. Whilst the mammalian brain constantly produces new neurons in certain areas, these cells are only incorporated in distinct brain areas and can not be recruited for repair of damaged brain tissue.

Immature neurons, so called neuroblasts, are generated by asymmetrical division of neuronal stem cells in the subventricular zone (SVZ) of the brain (Curtis et al. 2011). From there they follow migratory cues through a defined route, the rostral migratory

stream (RMS) through the ventral forebrain towards the olfactory bulb (OB) (Jankovski et al. 1998; Kirschenbaum et al. 1999; García-Marqués et al. 2010). The tube of surrounding glia cells together with blood vessels build up a physical boundary, separating the RMS from the adjacent tissue and hindering the migrating neuroblasts from escaping from their physiological path (Whitman et al. 2009). On route the neuroblasts interact with each other and form longitudinal aggregates, so called chains, which allows for a faster migration speed (Wichterle et al. 1997; Nam et al. 2007). Once the neuroblasts reach the OB, they separate from each other and migrate radially into the OB where they differentiate into granule and periglomerular cells (Mouret et al. 2009).
The polysialic acid (PSA) moiety is a posttranslational modification of the neural cell adhesion molecule (NCAM), highly expressed in migrating neuroblasts, consists of long, negatively charged chains of sialic acid (Finne 1982; Finne et al. 1983). The rapid reduction in NCAM-mediated interaction between cells is crucial for dynamic adhesive processes especially during development (Hildebrandt et al. 2007) but also in axon pathfinding of motor neurons (Landmesser et al. 1990; Tang et al. 1992, 1994), synaptic plasticity (Muller et al. 1996) and hippocampal learning processes (Becker et al. 1996; Senkov et al. 2006). Furthermore, PSA-NCAM plays a major role in the migration of postnatally generated neuroblasts. Both an NCAM-mutation as well as the enzymatic removal of PSA by endoneuraminidase-N (endoN) lead to a reduction of RMS migration whereas radial migration of individual cells remains unaffected (Ono et al. 1994; Hu et al. 1996; Chazal et al. 2000). In both cases not only fewer cells enter the RMS and the OB, also the structure of the RMS changes towards a widened and less organized form. A more recent study revealed a major difference between NCAM and PSA deficit: Apart from the aforementioned reduction of RMS-migration the enzymatic removal of PSA led to a dispersal of neuroblasts from the RMS into the surrounding tissue (Battista and Rutishauser 2010).
In the present study we utilized this effect to allow postnatally generated neuroblasts to leave their physiological route, the RMS, and test if these neuroblasts then migrate towards an excitotoxic brain lesion in the neighboring prefrontal cortex.

3.3 Material and methods

3.3.1 Subjects

This study was conducted on 33 adult (4.4 to 6.9 months) male Wistar rats (Charles River, Germany). The animals were kept under standard housing conditions (12 h light/dark cycle, lights on at 7 a.m., water *ad libitum*, standard lab chow 12 g/rat/day) in groups of 4 - 6 animals per cage. The experiments were performed in accordance with the National Institutes of Health ethical guidelines for the care and use of laboratory animals for experiments and were approved by the local animal care committee (Senatorische Behörde, Bremen, Germany).

3.3.2 Treatment

In a first stereotactic surgery the animals were lesioned by a bilateral microinjection of ibotenic acid (Cayman Chemical Company, MI, USA; 6.7 mg/ml saline; injection volume of 0.4 µl at 0.3 µl/min infusion rate) into the medial prefrontal cortex (rc. +3.2 mm; l ±0.5 mm; vd. -4.4 mm, relative to bregma). Control animals received a vehicle injection (phosphate buffered saline, PBS). In a second surgery five days later endoN (0.5 µl, provided by Prof. Dr. Gerardy-Schahn, Institute for Cellular Chemistry, Hannover Medical School) was injected into the lateral ventricle (rc. -0.8 mm; l ±1.4 mm; vd. -6.0 mm, relative to bregma) bilaterally at an infusion rate of 0,15 l/min. The enzyme was applied at concentrations of 50 µg/ml (endoN group), 500 µg/ml (endoN-Hi group) or vehicle only (control group). The resulting experimental groups were: vehicle / vehicle (C/C): n = 6; vehicle / endoN (C/E): n = 7; ibotenic acid / vehicle (L/C): n = 6; ibotenic acid / endoN (L/E): n = 8; ibotenic acid / high dose of endoN (L/E-Hi): n = 3. One of the L/E animals was excluded from further analysis, since the lesion could not be identified unequivocally.
The surgical procedures were performed under isoflurane anaesthesia (CP-Pharma, Germany; vaporizer in circle system), regulated to keep the spontaneous breathing rate of the animals at 40 - 60 breaths per minute. The injection cannulae (custom made from 30 G epidermal stainless steel cannulae) were left at the given coordinates for three minutes before and after each injection. Subsequently, the trepanations were closed with bone wax (SMI AG, Belgium). Before suturing the skin, an antiseptic ointment (Betaisodona, Mundipharma, Germany) and a local anaesthetic (Xylocain 2 %, AstraZeneca GmbH, Germany) were applied.
Three weeks after the second surgery the animals were euthanized by intraperitoneal injection of sodium pentobarbital (200 mg/kg bodyweight at a concentration of 20 mg/ml PBS; Sigma-Aldrich Chemie GmbH, Germany) and transcardially perfused with cold PBS, followed by 4 % paraformaldehyde solution.
The capability of the used endoN to eliminate PSA-NCAM from rat brain tissue was tested *ex vivo* beforehand. Single brain sections of perfused, otherwise untreated animals were incubated in a lower concentrated endoN solution (1 µg/ml) overnight. These sections were then stained for PSA-NCAM following the protocol described below.

3.3.3 Immunohistochemistry

Immunohistochemical double staining against the neuroblast marker doublecortin (DCX) and the marker for adult neurons, NeuN was performed on free floating 40 µm sagittal brain cryosections (distance between sections: 120 µm). After blocking in 10 % normal donkey serum (Jackson ImmunoResearch, PA, USA) and 0.05 % Triton-X for 1 hour at room temperature, the primary antibodies (Millipore Rabbit anti-NeuN (RRID: AB_10807945), 1:1000 and Santa Cruz Doublecortin Antibody (C-18) (RRID: AB_2088494), 1:1000 in the blocking solution) were allowed to incubate for 24 hours at 4° C. The sections were then rinsed and blocked in 10 % bovine serum albumin (Sigma-

Aldrich Chemie GmbH, Germany) before incubation with the secondary antibodies (CruzFluor 488 Donkey anti-Rabbit IgG (RRID: AB_10989100), 1:2000 and Santa Cruz Biotinylated Donkey anti-Goat (RRID: AB_631726), 1:1000)) for 24 hours at 4° C. Again, the sections were rinsed and blocked, then the streptavidin conjugated dye (Jackson Streptavidin-Alexa Fluor 568 (RRID: AB_2337250), 1:2000, in the blocking solution) was allowed to incubate for another 24 hours at room temperature. Before mounting and coverslipping (fluorescent mounting medium, Dako, Denmark) the specimen were counterstained for ca. 10 minutes in 0.9 % w/v Sudan black (Acros Organics, Belgium) in 70 % ethanol.
Previously endoN-incubated brain sections of untreated animals (see above), as well as exemplary sections from the experimental groups were stained for PSA-NCAM. The procedure followed the protocol described above, but the anti-NeuN antibody was exchanged for an antibody against PSA-NCAM (mab-735 (RRID: AB_2619682), provided by Prof. Dr. Gerardy-Schahn, Institute for Cellular Chemistry, Hannover Medical School), followed by a fluorescent coupled secondary antibody (Jackson Alexa Fluor 488 AffiniPureGoat anti Mouse IgG (H+L) (RRID: AB_2338840)).

3.3.4 Image acquisition and analysis

Images were acquired on a fluorescent microscope (Axioscope 100, Carl Zeiss AG, Germany) equipped with a monochrome digital camera (Spot, Visitron Systems GmbH, Germany). Further image processing was performed in the ImageJ (version 1.49h) (Schneider et al. 2012; Schindelin et al. 2015) based software FIJI (Schindelin et al. 2012). The overview images were then stitched in Microsoft Image composition editor (Microsoft Cooperation, version 1.4.4.0) using the planar motion 1 (rigid scale) setting. High-magnification images were taken as z-stacks (10 images each) and combined in FIJI, using the “extended depth of field” macro (Wheeler) with a radius set to 5 px.
The lesions and RMS were examined hemisphere-wise. If the structure of interest could not be identified in the available brain sections without doubt, the hemisphere was excluded from analysis. This resulted in a reduced sample size in the RMS measurements.
DCX-positive cells were counted on the sagittal slices approximately 1.4 mm lateral to bregma by an observer blind to the treatment. The area of the lesion was measured in the same brain section.
The width of the RMS was measured at three different positions (see Fig 3.1).
All statistical analyses were conducted in SigmaStat (version 3.5 for Windows).

3.4 Results

3.4.1 Ibotenic acid induced lesions

The injection of ibotenic acid into the prefrontal cortex reliably induced lesions of the tissue. A brain volume including parts of the prelimbic and infralimbic cortex and in some cases parts of the cingulate cortex, area 1 and the dorsal peduncular cortex was observed to be without NeuN-positive cells three weeks after the injections. We dis-

cussed the glial response to identically induced lesions elsewhere (Gundelach and Koch 2018). The vehicle injection caused no detectable lesion apart from the cannula channel.

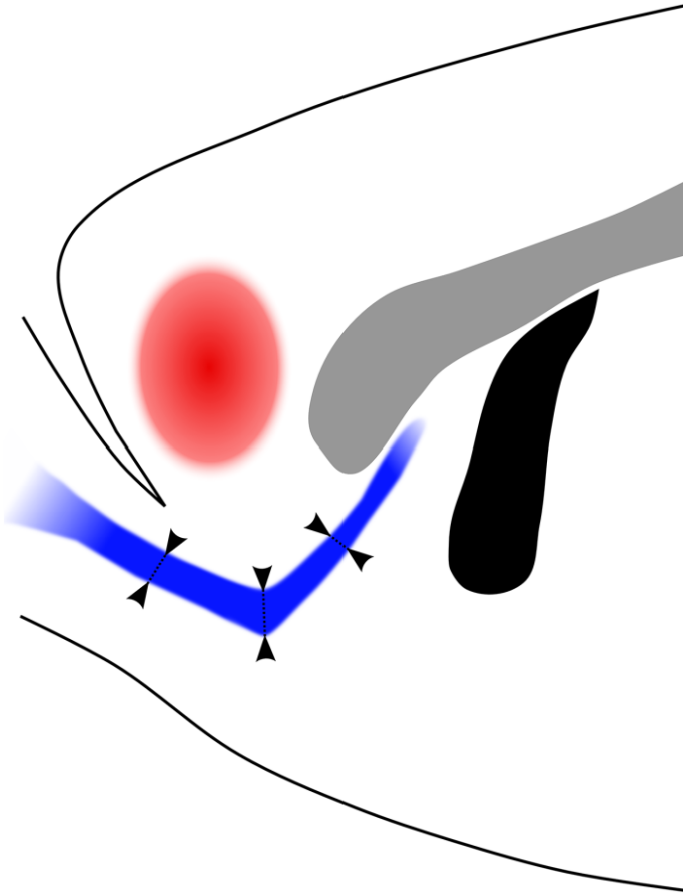

Fig 3.1: Schematic representation of the approximate position of the lesion and the RMS. The lesion is depicted in red, the RMS in blue. The extent of the lesion varied among subjects but was mostly limited to parts of the prelimbic and infralimbic cortex and in some cases parts of the cingulate cortex, area 1 and the dorsal peduncular cortex. The arrowheads mark the positions for the measurements of the rostral, ventral, and caudal RMS. Picture modified from (Paxinos and Watson 1998).

3.4.2 EndoN eliminates all PSA-NCAM *ex vivo* only

After an incubation period of 24 h in endoN of single brain sections virtually no PSA-NCAM was detectable by anti-PSA-NCAM staining (Fig 3.2 a and b). However, the brains of the study subjects showed some PSA-NCAM staining after the given survival time of three weeks. This staining was mostly limited to the SVZ and the RMS, whereas the surrounding tissue was only PSA-NCAM-positive in the vehicle-treated animals.

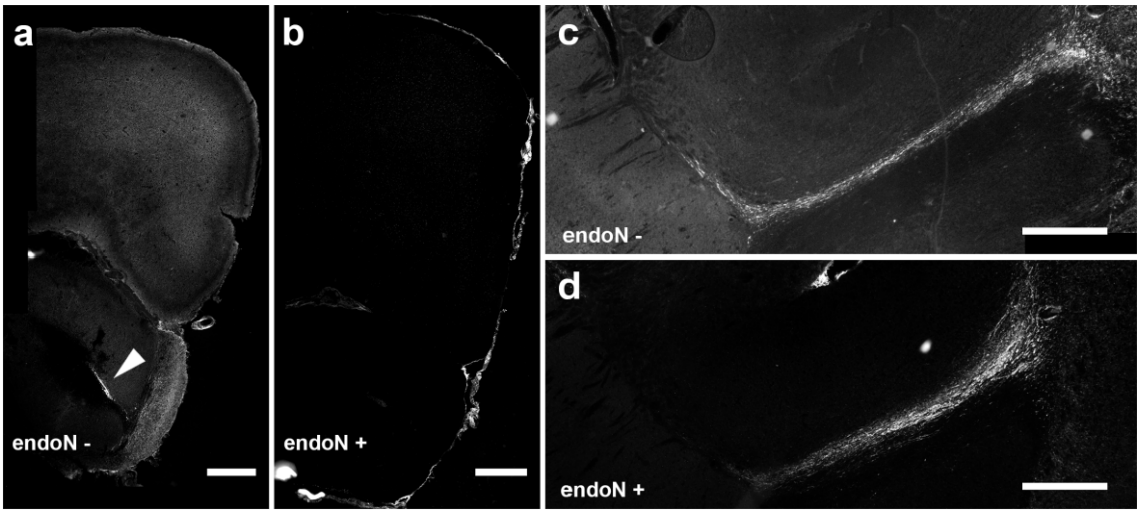

Fig 3.2: Effect of endoN-treatment *ex vivo* (a and b) and *in vivo* (c and d). Without endoN-treatment PSA-NCAM can be detected as diffuse staining throughout the whole brain (cp. (Bonfanti 2006)). Areas such as the RMS (ventral branch is marked by the arrowhead in (a)) or the SVZ and parts of the hippocampus show stronger staining for PSA-NCAM. After 24 h in endoN solution virtually no PSA-NCAM was detectable in the brain sections (b). After intraventricular injection of endoN and a survival time of three weeks only the weaker, diffuse staining was reduced, whereas the RMS appeared broader but with a strong detection of PSA-NCAM (c and d). Scale bars represent 500 µm.

3.4.3 Morphology of the RMS

As reported before (Ono et al. 1994; Hu et al. 1996; Battista and Rutishauser 2010), endoN application caused the RMS to become wider with a less organized structure. In order to evaluate the effectiveness of the endoN-treatment the width of the RMS was assessed.

Under physiological conditions the RMS shows a coherent path of DCX-positive cells, many of which form elongated aggregates (Fig 3.3 a and c). The application of endoN causes the RMS to become wider and more diffuse with a less uniform orientation of cells (Fig 3.3 b and d). Also the formation of cell chains is reduced. A change of general migration direction from the RMS towards the excitotoxic lesion was observed in the L/E and L/E-Hi groups but not the controls (Fig 3.3 e).

The measured RMS thickness varied between subjects and was likely also influenced by the exact anatomical position of the brain section examined. However, in the caudal and rostral portion of the RMS, the diameter differed significantly between treatment groups (analysis of variance after ln transformation, caudal $P = 0.004$; rostral $P = 0.006$). As revealed by the all pairwise *post-hoc* test (Student-Newman-Keuls) all endoN-treated groups differed from the C/C group significantly, both in the caudal (C/E ($n = 11$) vs C/C ($n = 8$): $P = 0.021$; L/E ($n = 10$) vs C/C: $P = 0.019$; L/Ehi ($n = 5$) vs C/C: $P = 0.013$) and the rostral (C/E ($n = 9$) vs C/C ($n = 10$): $P = 0.016$; L/E ($n = 7$) vs C/C: $P = 0.030$; L/Ehi ($n = 3$) vs C/C: $P = 0.012$) measurement point. In neither of both positions the

L/C group did not differ significantly from the C/C group (caudal: P = 0.359, n = 9; rostral P = 0.072, n = 8). In the ventral portion of the RMS no significant differences were found (P = 0.163; C/C: n = 7; C/E: n = 12; L/C: n = 8; L/E: n = 9; L/Ehi: n = 5).
Taken together, all endoN-treatments caused the RMS to become wider than the control treatment in the caudal and rostral, but not the ventral part of the RMS. The lesioned animals that did not receive an endoN injection (L/C group) did not show a significantly wider RMS in any position.

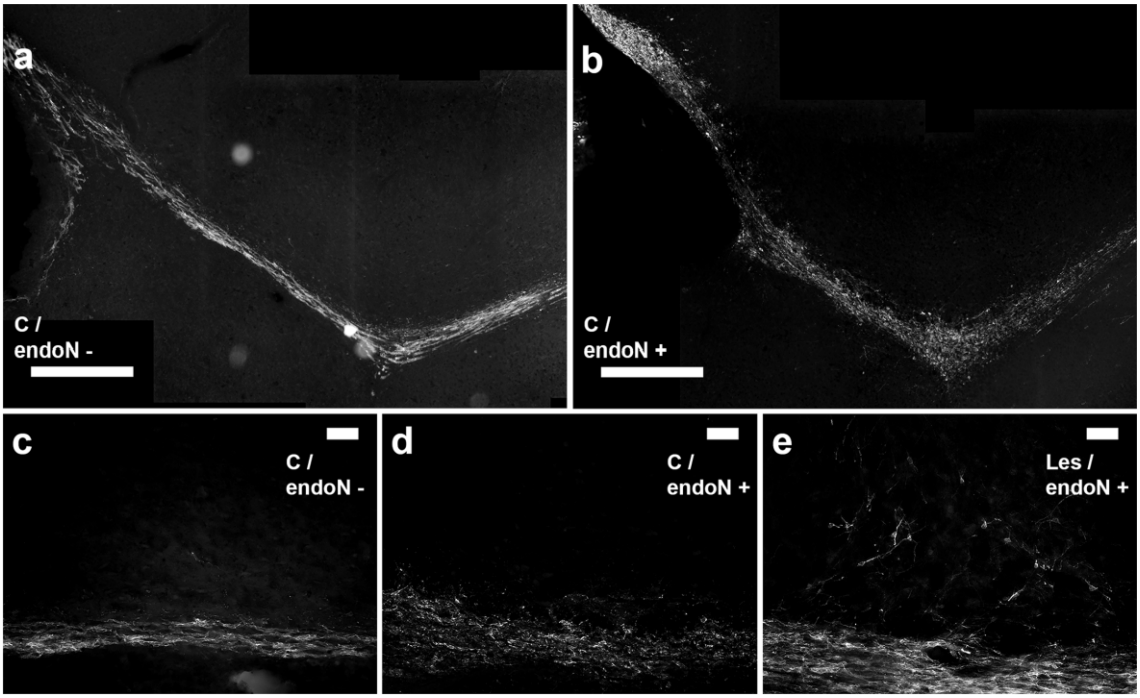

Fig 3.3: Effects of treatments on RMS morphology. DCX staining of the RMS of control animals (a and c), sham-lesioned and endoN-treated animals (b and d). Overall the RMS appears wider and less organized after endoN-treatment. c – e: Detail of the caudal, ascending portion of the RMS. Neuroblasts leaving the caudal RMS towards the excitotoxic lesion are shown in e (the lesion is located above the shown detail) compared to the equivalent part of the RMS of sham-lesioned animals after control (c) and endoN-treatment (d). Scale bars: 500 µm (a and b); 50 µm (c – e).

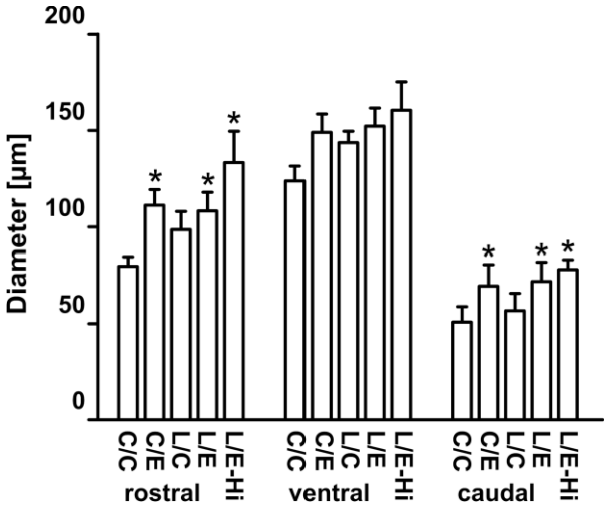

Fig 3.4: Diameter of the RMS measured at three different locations (s. fig 3.1). In the rostral and caudal measurements the endoN-treated animals (groups C/E, L/E, and L/E-Hi) differed significantly from the control animals (C/C; $P < 0.05$), but not from each other. Also the lesioned, but not endoN-treated animals (L/C) showed no significantly widened RMS compared to controls.

3.4.4 Two different populations of cells are positive for DCX

As discussed elsewhere (Kunze et al. 2015; Gundelach and Koch 2018), two different types of cells positive for the marker DCX are found in brain lesions. In short, one type shows strong immunoreactivity and the typical, polar morphology of neuroblasts (Martinez-Molina et al. 2011). The other cell type that is also positive for the astrocytic marker GFAP, has a larger soma with multiple processes and is only weakly stained by the DCX antibody (cp. (Gundelach and Koch 2018), Fig 3.2 g - i). Only the cell type described first was counted as neuroblasts.

3.4.5 Cells migrate into the lesion after endoN-treatment

Both doses of endoN resulted in a significantly higher number of DCX-positive neuroblasts within the lesion compared to lesioned and vehicle injected controls (Kruskal-Wallis One Way Analysis of Variance on Ranks; $H = 13.440$; 2 degrees of freedom, $P < 0.001$; see Fig 3.6). However, the effects of the doses were not different from each other with respect to the cell number. Neuroblasts were found in the lesions of 71.4 % of the endoN-treated animals ($n = 14$ hemispheres) and 100 % of the endoN-Hi group ($n = 6$ hemispheres). 25 % of the control lesions ($n = 12$ hemispheres) showed comparably low numbers of neuroblasts.

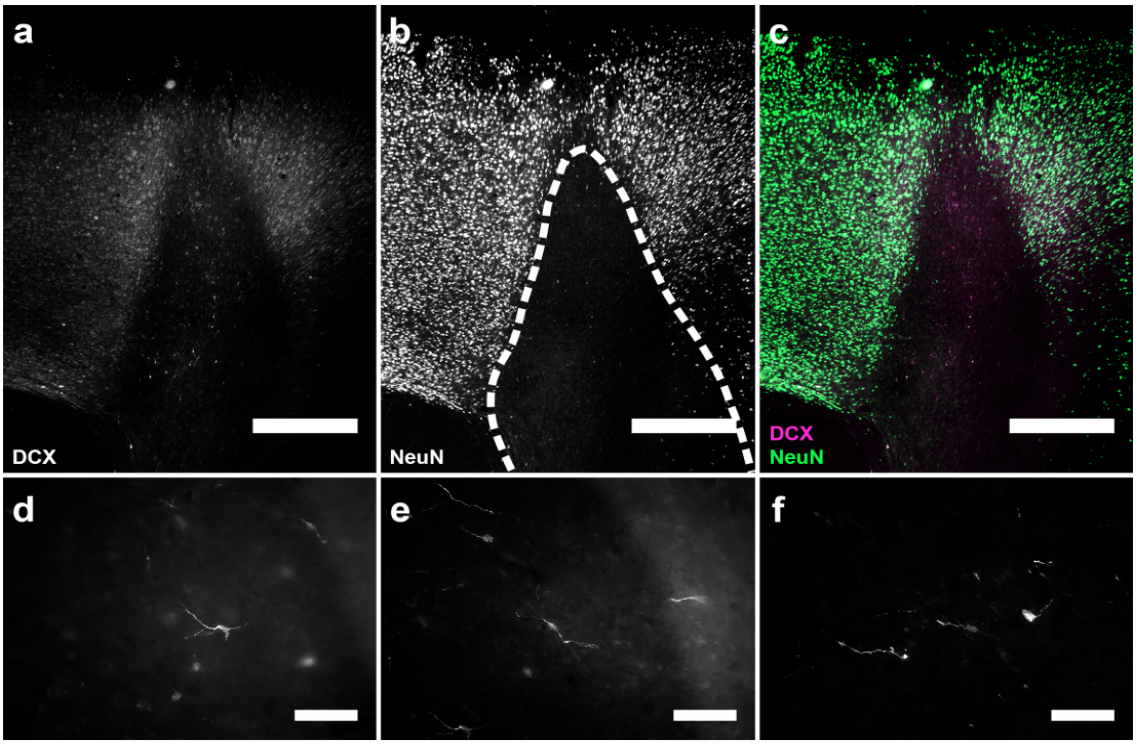

Fig 3.5: Neuroblasts within the lesion. DCX-positive cells (a) within the lesion (region without NeuN staining, circumscribed by dashed line in (b)), showing the typical morphology of migrating neuroblasts (cp (Martinez-Molina et al. 2011)). d – f show higher magnification photomicrographs of the relocated neuroblasts. All images depict sagittal plane, orientation: left: caudal, top: dorsal; scale bars: a – c: 500 µm; d – e: scale bars 50 µm.

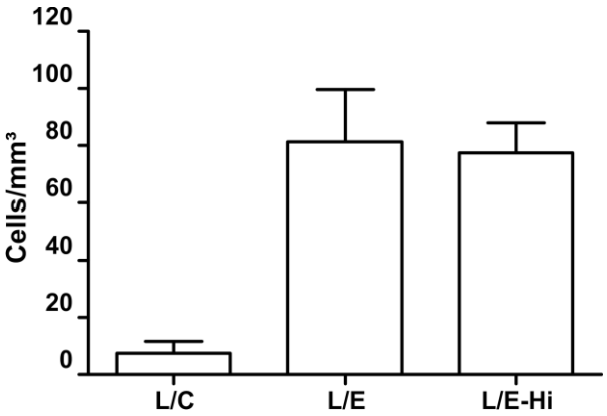

Fig 3.6: DCX-positive neuroblasts found within the lesion. Both doses of endoN (L/E and L/E-Hi) caused significantly ($P < 0.001$) more neuroblasts to migrate into the lesion than in lesioned and sham-injected (L/C) animals. The doses did not result in different cell numbers.

3.5 Discussion

In the present study we demonstrated that the known effect of endoN to allow the dispersion of SVZ-neuroblasts from the RMS, allows these cells to migrate towards a lesion in the vicinity of the RMS. After injection of two different doses of the enzyme into

the lateral ventricle of lesioned rats, significantly more DCX-positive neuroblasts were found within the lesioned tissue, compared to that of control animals.

3.5.1 Effect of endoN-Treatment

As revealed by the *ex vivo* experiment, endoN removed all detectable PSA-NCAM from free floating rat brain sections even at a lower concentration than used *in vivo*. Therefore, it was somewhat surprising to find PSA-NCAM in the RMS of endoN-treated animals. However, PSA-NCAM was mostly detected in the SVZ and the RMS, whereas the surrounding tissue showed little PSA-NCAM immunoreactivity.
Although it is possible that the endoN applied only had an incomplete effect, we hypothesize that the effect of endoN has rather decayed and the PSA-NCAM immunoreactivity has partially recovered until the end of the experiments. This assumption is supported by the lack of difference between the applied doses of endoN. In general, the enzyme is reported to cleave PSA-NCAM completely for a prolonged time of at least 7 days after intracranial injection (Ono et al. 1994; Burgess et al. 2008; Battista and Rutishauser 2010). In a study on young mice endoN was injected into the lateral ventricle at PD1 (Seki and Rutishauser 1998). Here, PSA-NCAM was found to be partially re-expressed after 30 days and almost fully restored after 1.5 months. On the other hand, another study found re-occurring PSA-immunoreactivity in parts of the hippocampus of rats after electric stimulation as early as five days after a single injection of endoN into the ventricle (Pekcec et al. 2007).
In the present study we also evaluated the effects of endoN application on the structure of the RMS. Although our study was not opted for the RMS measurements, which resulted in a low power of these data, the measurements indicate that the enzymatic treatment led to a widening of the caudal and rostral RMS compared to controls. Furthermore, the structure of the RMS of endoN-treated animals was less organized and the typical chains of neuroblasts were observed less frequently. These results go along with other studies in which endoN caused PSA-NCAM to become completely undetectable (Battista and Rutishauser 2010).
Taken together, the change in the RMS structure as well as the effect of endoN on migrating neuroblasts (see below), demonstrate the potency of the endoN injection in our experiments, although the effect on PSA-NCAM immunoreactivity was less pronounced than in other studies.

3.5.2 Effects of the excitotoxic lesion and endoN on migrating neuroblasts

Under physiological conditions neuroblasts are guided by chemoattractive and –repulsive signals towards their target locations (reviewed in (Sun et al. 2010; Leong and Turnley 2011)). However, also damaged tissue in the brain attracts migrating neuroblasts over a shorter distance. This has been described in studies of spontaneous neuroblast migration towards damaged tissue (Jin et al. 2003; Lee 2006; Kunze et al. 2015), but is also backed by our earlier work (Gundelach and Koch 2018), in which a known

chemoattractive substance, laminin, was used to redirect neuroblasts from the RMS. After the cells had left the artificial migration path, they kept on migrating in a now randomly oriented manner throughout the brain lesion.
Previous studies have demonstrated the importance of PSA-NCAM for the chain migration of neuroblasts tangentially through the RMS towards the olfactory bulb, whereas the radial migration of single cells seems to be largely unaffected by the lack of PSA-NCAM (Ono et al. 1994; Hu et al. 1996). However, a more recent study found PSA overexpression to enhance the sensitivity of single neural precursor cells towards various migratory cues (Glaser et al. 2007).
We demonstrated that SVZ-derived cells, once they have left the RMS, migrate towards a brain lesion despite the previous removal of PSA-NCAM. Since the migration of individual cells rather parallels the radial migration of neuroblasts in the OB, it is not surprising that this mechanism remains functional. However, our results also show that the migratory cues released by the damaged tissue are capable of directing the neuroblasts over a distance of several millimeters. This finding verifies that the migration of neuroblasts towards lesion sites is based on chemokines (Belmadani 2006; Robin et al. 2006; Yan et al. 2007), rather than the PSA-regulated PI3K receptor (Glaser et al. 2007).

3.5.3 Fate of relocated cells

In our present study, no cells both immunoreactive for DCX and NeuN were found within the brain lesions. This stands in contrast to our previous work on relocated neuroblasts in which a small number of cells showed morphological and immunological signs of differentiation (Gundelach and Koch 2018). It is possible that the relocated cells need more than the provided survival time of three weeks to migrate and differentiate. However, under physiological conditions most neuroblasts develop into OB-neurons within 15-30 days after proliferation (Petreanu and Alvarez-Buylla 2002). Interestingly, endoN-based removal of PSA from NCAM has been demonstrated to promote cell differentiation of neuroblastoma cells (Seidenfaden et al. 2003) as well as SVZ-neuroblasts: After demyelination of the corpus callosum, endoN-treatment not only enhances migration of SVZ cells towards the lesion, but also causes the cells to differentiate towards a oligodendroglial cell type (Decker et al. 2002). Furthermore, neuroblasts that do not leave the SVZ after endoN-treatment were found to differentiate towards a neuronal morphology and express tyrosine hydroxylase, resembling OB cells (Petridis et al. 2004). *In vitro* experiments revealed that this effect is cell-cell contact dependent and can be reduced by anti-NCAM antibodies. However, tyrosine hydroxylase expression was not detected in the cultured cells (Petridis et al. 2004). Together these findings indicate that the fate of neuroblasts can be largely influenced by the interaction with the surrounding tissue. Physiologically, these influences on proliferating and migrating neuroblasts are prevented by the polysialated NCAM within the cell membranes. Once the cells reach their position within the OB, PSA-NCAM is downregulated (Rousselot et al. 1995), thus a similar, contact dependent fate determination is conceivable. In our experiments a large volume of the surrounding tissue was lesioned, thus reducing the potential

of interaction between neuroblasts and intact nerve cells. Also the neuroblasts were found to spread within the lesion, which makes an interaction between these cells and an endoN-induced cell differentiation unlikely.

3.5.4 Potential for the development of a clinical application

The long term objective for the present study is to evaluate the endoN-induced neuroblasts migration towards a brain lesion in the context of a clinical application after structural brain damage in humans.

The general existence of postnatal neurogenesis in the human SVZ and hippocampus are well established (Curtis et al. 2011), however, there are differences between the animal model and humans. The knowledge on human neurogenesis and neuroblast migration is widely based on data from elderly subjects, since only post-mortem analysis is applicable. Furthermore, the widely used protein markers for developing neuronal cells can not always be interpreted unequivocally. This is not only the case for DCX-positive astrocytes (Kunze et al. 2015; Gundelach and Koch 2018), also markers for adult stem cells are expressed by a non-proliferative cell type (Gebara et al. 2016). Therefore, cells often need to be identified by the co-expression of multiple markers or by the cell morphology. These limitations make the transfer to the human brain especially challenging. Recently, the persistence of neurogenesis throughout adulthood has been challenged (Sorrells et al. 2018) which has kindled a discussion about neurogenesis in the adult human brain (Snyder 2018). Apart from the recent debate, it is known that the initial generation of new neurons in the SVZ and hippocampus declines after infancy (Sanai et al. 2011; Bergmann et al. 2012). A similar, yet less pronounced decline has also been found in rodents (Kuhn et al. 1996; Tropepe et al. 1997; Shook et al. 2012). A better understanding of this age related change might eventually provide methods to sustain neurogenesis and neuroblast migration which in turn would provide new perspectives for clinical treatment of brain damage, based on endogenous neuroblasts.

In order to develop a viable treatment for structural brain damage, further conditions need to be fulfilled. Our data demonstrate the capability of SVZ-derived neuroblasts to migrate towards a brain lesion spontaneously, probably guided by chemoattractive cues (Fallon et al. 2000; Belmadani 2006; Robin et al. 2006; Yan et al. 2007; Courtès et al. 2011). It needs to be evaluated over what distances these cues affect neuroblasts after they were enabled to leave their predetermined path. Furthermore, the long term fate of re-located neuroblasts needs to be addressed. For example the inhibition of programmed cell death (Gascon et al. 2007; Kim et al. 2007) might increase the number of neuroblasts within the lesion. Furthermore, we found no signs of newly differentiated neuronal cells within the lesions three weeks after the endoN-treatment, which means the previously described differentiation stimulating effects of endoN (Petridis et al. 2004) do not show under the given experimental conditions. However, there are molecules known to promote differentiation of neuronal precursor cells, such as brain derived neurotrophic factor (Ahmed et al. 1995), epidermal growth factor (Reynolds and Weiss 1992), insulin-like growth factor-I (Arsenijevic and Weiss 1998), and leukemia inhibitory factor

(Memberg and Hall 1995). Application of these substances to relocated neuroblasts might enable their differentiation and integration into the surrounding neuronal tissue eventually.

3.5.5 Conclusion

The endoN-based technique enables migration of neuroblasts into a brain lesion and utilizes the migratory cues probably released by the lesion to guide the neuroblasts to their target. Therefore, it requires low surgical effort. However, the neuroblasts do not seem to differentiate within the lesion spontaneously. Furthermore, it is unclear to what extent neurogenesis declines after infancy in humans. It remains for future studies to determine if endoN might prove useful for the development of a future clinical treatment for structural brain damage.

Acknowledgments

We thank Prof. Dr. Gerardy-Schahn and Prof. Dr. Hildebrandt for generously providing endoN and the antibody against PSA-NCAM as well as valuable information about the substances. Furthermore, we thank Maja Brandt for excellent technical assistance.

4 Experiments that were not included in the publications

4.1 Magnetic resonance imaging

Initially, repeated magnetic resonance imaging (MRI) was planned in order to evaluate the longitudinal effects of the excitotoxic brain lesions and the potentially counteracting treatments. However, since this technique yielded no usable data in the study's context, the MRI scans were excluded from the final experimental design.

4.1.1 In a nutshell: Diffusion Tensor Imaging (DTI)

DTI is a special sub discipline of MRI and is used in clinical context to identify brain lesions (Gupta et al. 2008) and investigate structural connectivity (Nucifora et al. 2007; Gong et al. 2009). In order to detect water diffusion, two diffusion weighting gradient pulses are applied symmetrically before and after the 180 degree refocusing pulse within a DTI sequence. Within stationary molecules the effects of both gradient pulses cancel each other out. However, if the molecules moved within the period of time between the pulses, they stay dephased. Thus, the acquired signal will be attenuated proportionally to the degree of water diffusion in the direction of the diffusion weighting gradient (Alexander et al. 2007).

By application of several gradient directions, the amount and direction of water displacement within each image voxel can be reconstructed by comparison of each diffusion weighted volume to a reference volume, acquired without diffusion weighting gradient, the so called A0 image set.

The resulting information about the diffusion within each voxel, the diffusion tensor, can be visualized as an ellipsoid (Fig 4.1) as postulated by Basser et al. (1994). The polar axis of this ellipsoid represents the major direction of gross diffusion within the voxel and is thus oriented according to the tensor's main eigenvector and scaled by the main eigenvalue λ_1. The lengths of the ellipsoid's smaller radii are defined by the minor eigenvalues λ_2 and λ_3, and therefore represent the level of non directed diffusion.

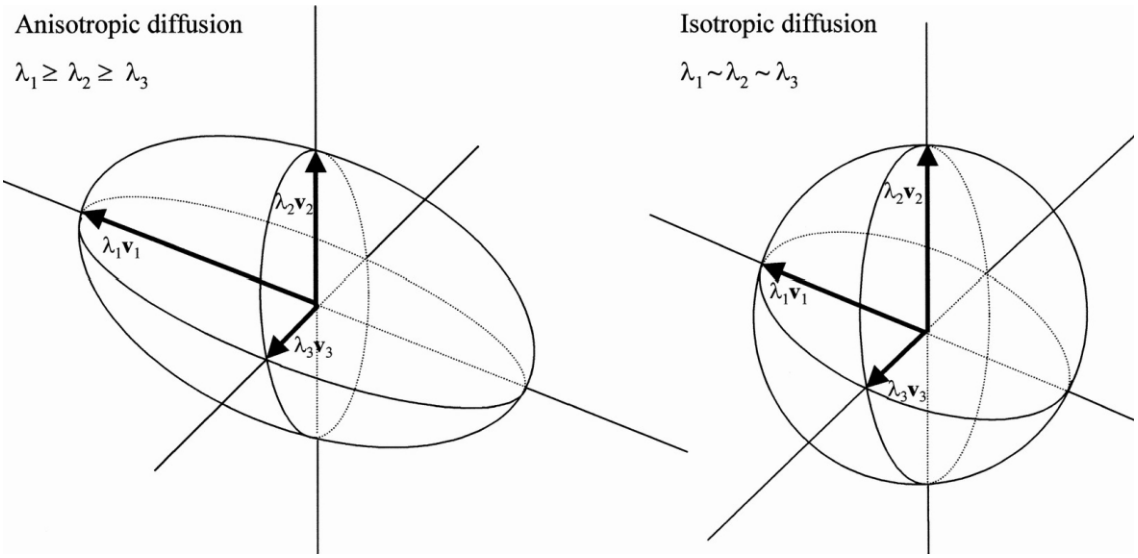

Fig 4.1: Diffusion ellipsoids for anisotropic and isotropic diffusion. In case of isotropic diffusion (i.e. unrestricted diffusion), the eigenvalues (λ) are about identical. If water displacement is hindered by physical boundaries, diffusion is oriented, i.e. anisotropic. In this case λ1 is larger than the eigenvalues of the perpendicular directions. Illustration adapted from Wiegell et al. (2000).

The method of DTI relies on the gross amount and anisotropy of water diffusion within different biological tissues. Generally, diffusion processes are driven by Brownian motion and hence randomly oriented. Therefore, a tensor ellipsoid, acquired within a structureless probe, would be spherical indicating equal amount of gross diffusion in all directions. In biological tissue such as nervous structures, but also layered organs like muscles (Beaulieu 2002), or botanic tissues such as vascular bundles (Le Bihan et al. 2001), molecule displacement is hindered by physical boundaries. For example molecules within and between the axons of a fiber bundle will travel further along the bundle's direction than perpendicular to it.

Consequently, DTI allows interpretation of properties of a sample below the physical resolution of the scan. The DTI contrasts most relevant for the present study were:

The Trace of diffusion (T(D)),

$$T(D) = \frac{\lambda_1 + \lambda_2 + \lambda_3}{3}$$

which describes the total level of diffusivity within a volume.

The fractional anisotropy (FA) (Basser and Pierpaoli 1996),

$$FA = \sqrt{\frac{1}{2}} \frac{\sqrt{(\lambda_1 - \lambda_2)^2 + (\lambda_1 - \lambda_3)^2 + (\lambda_1 - \lambda_3)^2}}{\sqrt{(\lambda_1^2 + \lambda_2^2 + \lambda_3^2)}}$$

which describes a ratio of directed diffusion to the overall diffusion within the volume.

Furthermore, the A0 image and the trace weighted image (TWI) were qualitatively analyzed. The A0 image represents the signal intensity without the diffusion gradients, whereas the TWI represents a direction independent diffusion contrast. It is calculated as follows:

$$TWI = A0 \cdot \exp(-b \cdot T(D))$$

where b is defined as a fixed value of 1000 s/mm².

Alterations in T(D) are associated with structural tissue damage since water can diffuse easier (for example due to cell loss) or less easy (as observed in later stages after intercerebral hemorrhage (Kang et al. 2001)). The FA, on the other hand, is especially sensitive for disturbances in white matter and is used to diagnose fiber tract damage or demyelination for example in multiple sclerosis (Sbardella et al. 2013). The A0 image reflects the T2-signal intensity and is therefore determined by the water content of the tissue. The TWI adds information about the water diffusion to these images. Both the A0 and TWI volumes can be useful for side by side visual inspection. However, since the signal intensity is also influenced by factors such as temperature, distance of the specimen from the receiver coil, or inhomogeneities of the magnetic field, these measures may be offset from scan to scan. Thus, these images are not suitable for a voxelwise comparison without prior normalization steps.

DTI was included in this study design to spatially quantify the brain lesions due to an area of increased gross diffusion and changes in FA. If relocated neuroblasts caused a reinnervation of the lesioned area, the overall diffusivity in this area would decline in later scans.

4.1.2 Data acquisition and processing

Data Acquisition

A total of 14 adult male Wistar rats were included in the MRI experiments. 10 rats underwent the surgical procedures described in the laminin study (2.3.2), resulting in 4 lesioned/control animals and 6 lesioned/laminin-treated animals. Additionally 4 animals were sham-lesioned and sham-laminin-treated to serve as comparison. Subjects were then scanned 1, 15, or 29 days after the second surgery, or at multiple of these time points, resulting in a total of 28 scans. Within the imaging system (7 tesla Bruker Biospec 70/20) the animals were anesthetized with an isoflurane/oxygen mixture (0.5 to 4 %; regulated to maintain a respiratory rate of 40 to 60 breaths per minute) and kept on a heated bed set to a water temperature of 35 °C. DTI images were acquired using an echo planar imaging (EPI) sequence with a diffusion weighting gradient duration of 4.0 ms and an effective B-value of 1000 s/mm². Readout was performed at a bandwidth of 250 kHz at an echo time (TE) of 33.34 ms. Repetition time (TR) was set at 6000 ms. 30 diffusion directions and 5 A0 images were acquired with an averaging factor of 4 at a matrix size of 128 x 86 x 23 voxels, resulting in an anisotropic voxel resolution on 234 x 233 x 450 µm/voxel (rostro-caudal x lateral x dorso-ventral). Additionally, a T2 weighted TurboRARE structural scan was acquired (TE: 12.0 ms; TR: 8000 ms; RARE-factor: 8). The acquired matrix of 256 x 384 pixels resulted in a resolution of 117 x 118 µm (lateral x rostro-caudal) at a slice thickness of 500 µm.

Data pre-processing

The MRI data was reconstructed in ParaVison 5.1 (Bruker BioSpin, USA), which was also used to calculate the DTI contrasts (FA, T(D), A0, Trace weighted image, as well as the tensor eigenvectors and eigenvalues). Next, the volumes were imported and split into FIJI by a custom written plugin (Bruker DTI opener v 1.1.3). The plugin interprets Bruker's data structure in order to read image sets correctly, but does not alter the image content. The image dimensions were then multiplied by a factor of 10 to enable SPM 12 (Friston et al. 2007), which is designed to work on human brain scans, to process the rat brain data.

Spatial normalization

In order to evaluate the rat brain volumes on a voxel based level the volumes have to be spatially normalized to a standard brain template first. This process ensures that anatomical structures are located at identical image coordinates within each data set. Within an ideal brain scan this process would require some rotations, translations and possibly scaling. Unfortunately, fast imaging techniques required for DTI, such as the EPI-technique used in these experiments introduce a number of artifacts into the scan (Le Bihan et al. 2006). Some of these artifacts, such as ghosting, can be eliminated by optimization of the imaging parameters and the application of saturation slices. However, an inevitable problem in EPI is distortion which causes spatial artifacts of the image data unevenly throughout the scanned volume (see Fig 4.2). Therefore the co-registration process has to perform the more complex task of nonlinear warping in order to align a brain scan to a template.

The spatial normalization was performed in SPM 12. First, the A0 volume was manually pre-oriented to match a rat brain template (Valdés-Hernández et al. 2011). Furthermore the image origin was placed at *bregma*. The resulting reorientation matrix was then applied to the other DTI volumes. The spatial normalization was conducted by means of the "Normalise: Estimate" module, using the parameters given in Tab 4.1. Again, the resulting reorientation matrix was applied to all other DTI volumes of the same scan.

Tab 4.1: Parameters used in SPM 12's "Normalise: Estimate" module

Estimation option:	Setting:
Bias regularization	0.0001
Bias FWHM	30 mm cutoff
Affine Regularization	No regularization
Warping Regularization	0; 0.001; 0.001; 0.001; 0.001
Smoothness	0
Sampling distance	1.5

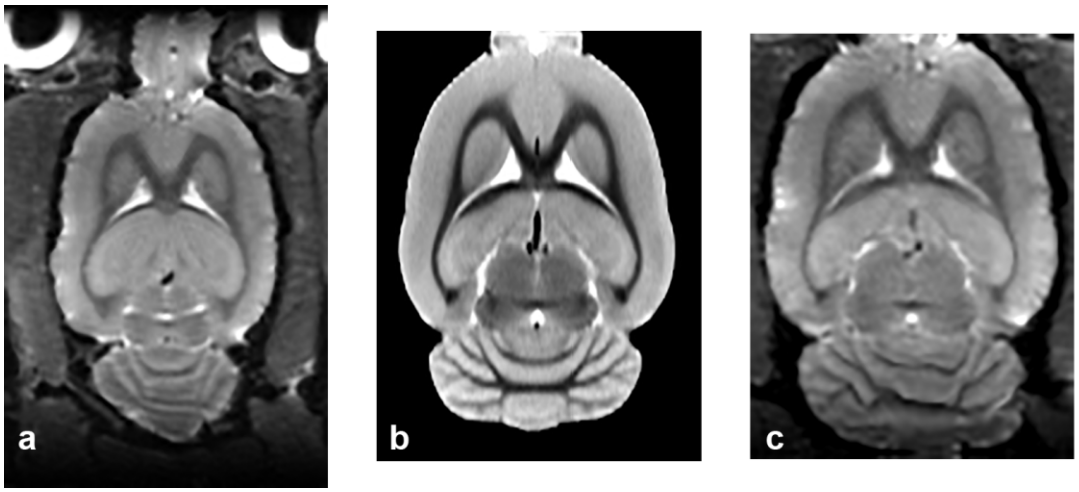

Fig 4.2: Normalization of A0 image to template. A single horizontal plane of an EPI-volume before (a) and after (c) normalization to the template volume (b). The procedure not only corrected the slight tilt of the volume (rostral part of the volume was tilted to ventral), but also removed the EPI-distortions (especially prominent in the left side of the plane) almost completely.

Statistical analysis

The statistical analysis of the co-registered volumes was also performed in SPM 12. After estimation of the general linear model, the t-contrasts were calculated on smoothed DTI volumes (gaussian, filtersize: 4 voxels, sigma: 4), with proportional normalization activated. The resulting statistical parametric maps were saved as nifti files and inspected in FIJI.

Only FA and trace maps were included in the statistical analysis, since these measures are scaled between 0 and 1 and therefore allow a direct comparison. The other DTI maps, such as the trace weighted image, or single diffusion components are directly influenced by parameters such as temperature or distance of the receiver coil to the rat's brain and would require further scaling measures prior to statistical processing.

4.1.3 Outcome of the MRI experiments – and why it did not yield useful data

Analysis 1: Voxelwise group analysis of normalized brain scans

In order to detect differences in the lesion-induced diffusivity between the treatment groups and the time after surgery, a voxelwise analysis of the scans was conducted initially.

A side by side comparison of the scans of the control animals demonstrated a successful normalization procedure. The outlines of the brain as well as its major structures were located congruently within the data sets with a variation of approximately ±1 voxel (Fig 4.4 a - c).

However, the statistical analysis yielded unclear results. In none of the analyzed DTI measurements differences above background level could be detected in the lesioned areas. This means although the lesions were visible in the DTI volumes of single scans, no group effects, neither due to treatment, nor time point could be detected.

The main reason for this finding lies in the variations of lesion location and extent between subjects. The voxel-based analysis described above requires the compared tissues to be congruent with each other among the brain scans. Surprisingly, the ibotenic acid induced lesions showed a remarkable variation in size and orientation (Fig 4.4 e and f). The post-mortem histology confirmed that this variation was not an MRI-artifact but inherent to the method applied. The reason for this variation in lesion location might be the comparably high volume of injected ibotenic acid and its diffusion pattern. Possibly a series of multiple injections of smaller volumes would have allowed more control over the lesion extent (cp. (Jarrard 1989)). However, the variations in lesion location achieved with the applied method did not allow for a voxelwise analysis of the normalized brain scans.

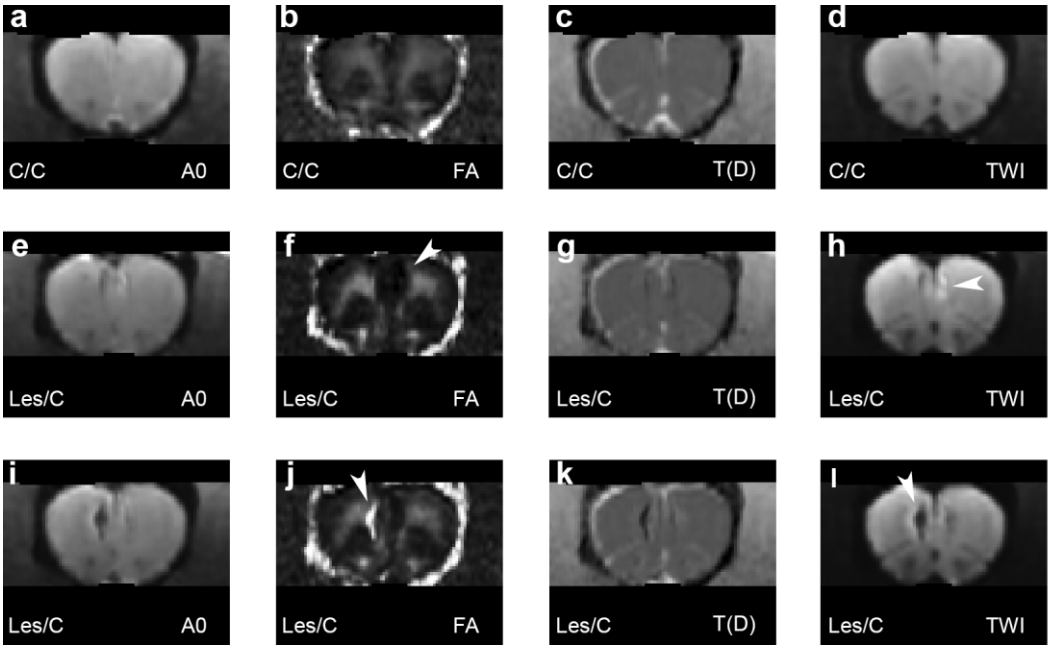

Fig 4.3: Examples of the obtained DTI contrasts (left to right: A0, FA, T(D), trace weighted image (TWI)). The upper row (a - d) show a non-lesioned control animal, the middle and lower row show scans of a lesioned animal 1 day (e - h) and 15 days (i - l) after the sham-laminin treatment, respectively. The lesion is most obvious as an area of decreased FA (arrowhead in f) since water diffusion is less directed after the loss of cells. Also an increase in overall diffusion is found (arrowhead in h) although this effect is non-uniform throughout the lesion. At the later time point a hemorrhage within the lesion is found. This is characterized by an area of restricted diffusivity (arrowhead in j and l).

Analysis 2: Within subject longitudinal analysis

In order to take the variation in lesion location between subjects into account, a qualitative longitudinal within-subject analyses was conducted. The aim was to characterize the changes of brain lesions over time and possibly detect differences between lesioned and laminin treated groups from lesioned and vehicle treated animals.

Once again, the results were inconclusive. The analysis revealed differences between measuring time points predominantly at the lesion borders. However, these effects were paralleled by differences at the borders of the ventricles. A closer inspection of the scans of the lesioned animals revealed inconsistencies in their normalization: As stated above, the optimization of the normalization procedure was conducted on control animals without brain lesions. However, major anatomical anomalies like the extensive brain lesion seen in this study severely interfered with the normalization method used by SPM (Fig

4.4). This means not only the overall outcome of the normalization might be less than optimal, but also the extent and position of the brain lesion might be severely altered by the geometrical transformation, since the software is not able to find a match for the lesioned tissue within the template. Consequently, it is unclear if any differences between measuring time points reflect anatomical changes or just an artifact of the normalization procedure. Therefore, the analysis was discarded.

In order to achieve better normalization results in lesioned brains, certain parts of the volume would have to be blocked from the comparison to the template brain. Still, this would yield imperfect results since distortions within the lesion and the surrounding tissue could not be compensated for, but at least this approach would not introduce new spatial artifacts.

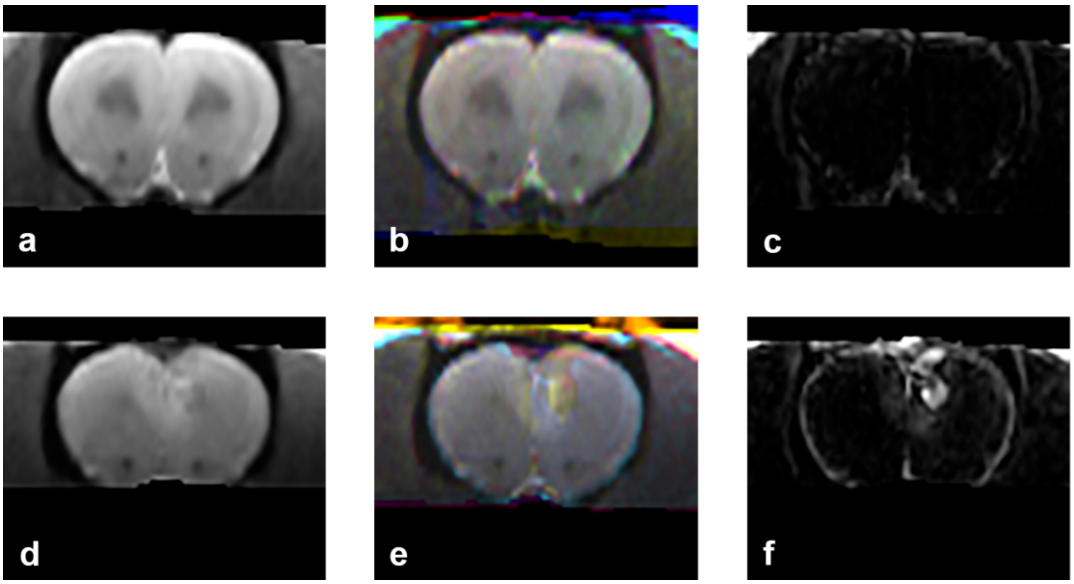

Fig 4.4: Assessment of normalization quality of sham lesioned (upper panel) and lesioned (lower panel) animals. Single, spatially corresponding frontal slices of normalized EPI-volumes of 8 scans of control animals and 6 of lesioned animals were overlayed. (a) and (d) show the averaged image stacks. The color coded overlay (each color representing one scan) shows that the control volumes are well aligned and the minute differences between the image slides are located in the dorsal- and ventralmost border regions of the brain (b). This is also visible in the standard deviation map of the stack (c): Only the mentioned border regions show an increased standard deviation . Much in contrast the overlays of the lesioned brains show a higher variance between the scans (e and f). This is mainly caused by the non-uniform localization of the brain lesions and further enhanced by lesion induced normalization artifacts. The high standard deviation zone around the perimeter of the brain (f) is a further indicator for an inferior normalization quality compared to the control group (c).

Analysis 3: Region of interest (ROI) based analysis

Additionally, in an attempt to compensate for variations in lesion location a ROI based analysis was performed. For this method the ROIs were manually drawn into the brain scans layer by layer and the FA and T(D) within the lesion were measured in FIJI. Additionally, these ROIs were averaged and overlayed to the scans of the sham lesioned animals. The data of these measurements served as a control. Statistical analysis of these data was then performed in Sigmastat (version 3.5 for Windows; SPSS Inc., USA).

The statistical analysis revealed significant differences between the lesioned and control scans (two-way ANOVA with Tukey post hoc test: C/C vs Les/C and C/C vs Les/Lam: $P < 0.001$), but not between the Les/C and Les/Lam groups ($P = 0.307$).

As revealed by the histological examination only a low number of cells was relocated in the lesions of laminin treated animals. Also, only single cells seemed to interconnect with the surrounding tissue. This means the originally hypothesized reinnervation of the lesioned tissue after relocation of neuroblasts did not take place. In order to produce visible effects in DTI, the formation of a dense tissue would be needed. Furthermore, the lesion induced gliosis, as well hemorrhages along the injection channels, caused a substantial variance of diffusivity within the brain lesions that would probably mask the effects of a hypothetical reinnervation.

For this reason also the ROI based analysis did not show any differences between the treatment groups. Since the data is measured over the full lesion volume, potential changes on single cell level could not be detected by this method. Furthermore, the method is prone to error, since the ROI has to be drawn manually.

4.1.4 Conclusion

While DTI is a proven tool in diagnosis of brain lesions in clinical context, unfortunately, our results revealed that it is not ideal for the study at hand.

Nonetheless, further insight into the potential treatment of structural brain damage could be achieved by an MRI-based longitudinal study. However, the technique included in the initial study design was planned under the assumption that relocation of neuroblasts would be succeeded by a substantial reinnervation of the tissue. Even if this assumption was true, the method at hand would need further refinement: The widely used spatial normalization methods align the scanned brain to a predefined standard brain. This process is less then optimal for brains that show large-scale deformations such as the lesions in this study. By trying to match the deformed brain to the template, the normalization algorithm obscures the true spatial properties of the lesion. Possibly, a more advanced method would allow the manual exclusion of certain areas of the brain scan in the normalization process and interpolate the necessary spatial alignment *post-hoc*. Alternatively, a different MRI method, less susceptible to deformation artifacts could be applied. Then again, these acquisition technique are usually slower and therefore not

suitable for diffusion based imaging. A promising approach is the labeling of single cells with iron oxide nanoparticles (Hoehn et al. 2002; Shapiro et al. 2007; Andreas et al. 2012; Li et al. 2013). This method allows the detection of small numbers of cells and their movement over time. Furthermore, a combined assessment of cell markers with diffusion based methods would allow discrimination of reinnervation processes from gliosis and subsequent glia scar formation. However, the targeted application of the nanoparticles to SVZ-neuroblasts as well as potential neurotoxic side effects present new challenges.

4.2 Behavioral experiments

In order to test for the effects of relocated neuroblasts on deficits caused by the prefrontal cortex lesion a number of behavioral experiments was performed on an early cohort of animals. Due to the unclear results of these experiments but also with respect to the histological analysis, which revealed a low number of relocated cells, the experiments were dropped from the final study design.
The conducted tests, as well as their outcome will be briefly presented here.

4.2.1 T-Maze alternation task

A test for spatial working memory deficits is provided by the alternation T-maze task (for a detailed protocol see (Deacon and Rawlins 2006)).
The test utilizes a T-shaped maze (arms 60 cm in length, 15 cm in width and surrounded by 30 cm high walls) consisting of a start compartment at one arm and to reward arms with built in food bowls. The start compartment is separated from the rest of the maze by a guillotine door. In each run the rat is placed in the start compartment. The guillotine door is then opened and the rat is allowed to enter one of the reward arms, before being removed from the maze.
During the training phase the rat’s innate tendency to alternately choose the left and right arm was reinforced. For this purpose only the arm opposite to that last entered by the animal was rewarded with two food pellets (45 mg Dustless Precision Pellets, Bio-Serv, UK). Training consisted of one daily session with 31 runs (one free choice run in which both arms were rewarded and 30 runs with a reward in one arm only) and was

conducted until the rat showed a performance above 80 % correct runs for three consecutive days.

During the test phase the animals had to wait for a distinct period of time (0, 10, or 30 seconds) until the guillotine door was lifted. The score of correct runs in percent was assessed for each delay.

As revealed by a two-way repeated measures ANOVA (Sigma Stat 3.5, SPSS Inc., USA) significant differences were only present between the delays (P = 0.005) but not between groups (P = 0.300) (see Fig 4.5). As expected, after both delays the animals performed significantly worse compared to the 0 second delay (Tukey test; 10 s delay: P = 0.031; 30 s delay: P = 0.006). No adverse lesion-effect was detected.

It remains unclear whether and to what extent the unclear results can be attributed to the rather low sample size (C/C: 7; C/Lam: 11; Les/C: 5; Les/Lam: 8; Les/LamHi: 5). Furthermore, the differences in size and location of the lesion described above (cp. Fehler: Verweis nicht gefunden) might have contributed to the variance of the animals' performance. It has been demonstrated that smaller excitotoxic lesions of a sub-portion of the medial prefrontal cortex, the anterior cingulate area, only cause transient deficits in the T-maze alternation task (Sánchez-Santed, De Bruin, Heinsbroek, & Verwer, 1997). It is therefore conceivable that not all lesions led to comparable results in this test, dependent on the parts of the prefrontal cortex affected.

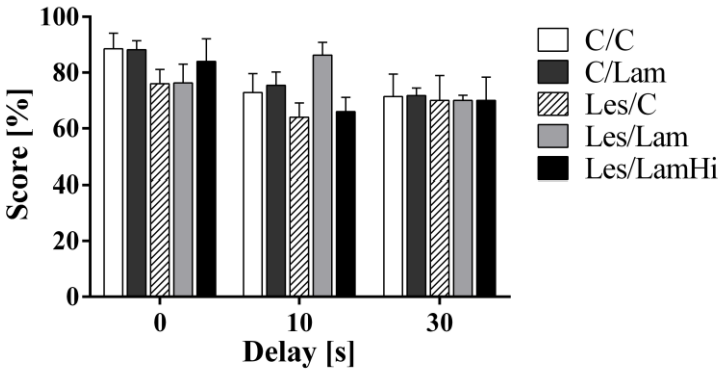

Fig 4.5: Results of the T-maze alternation task. Significant differences were only found between the delays (two-way repeated measures ANOVA: P=0.005) but not between groups (P=0,300).

4.2.2 Object recency task

The object recency task (Mitchell and Laiacona 1998) is a variation of the object recognition task which evaluates if the animal is able to remember which one of two familiar objects it has encountered further ago.

The experiments were conducted in plastic boxes (48 x 48 x 48 cm) which the animals were allowed to freely explore for 10 minutes. During two sample trials a set of two objects[2] (set A) was presented to each rat for five minutes. One hour later, another set of objects (set B) was presented to the animal for another five minutes. The test trial was conducted 22 hours later and consisted of another object-pair presentation. The object set during this phase consisted of one item from set A and another one from set B. The time the animals spent exploring each item was assessed *post hoc* via video analysis. Statistical analysis was performed on the time the animals spent exploring the two objects (in seconds). Also the percentage of time exploring each object was calculated to take individual differences in overall exploring time into account.

Although all treatment groups spent more of their exploring time with the less recent object, these differences were only significant within the Les/LamHi group (t-test: $P = 0.008$; Fig 4.6).

Apart from the relative low number of animals per group, the different objects used might contribute to individual differences in exploration time. The objects have been carefully selected to approximately match in size and have no chewable edges or openings through which the rats could stick their heads. Nonetheless, an object might appear more interesting to a rat than another, regardless of the period of time until it has been explored last. A selection of even more similar objects might have allowed the detection of the effect of object recency on the exploration time and possibly also a lesion effect. However, it has to be ensured that the objects can be identified and recognized by the rat.

2 Available objects: 20 ml glass vial with lid; case of Digital Video-cassette; lid of plastic jar, appr. 10 cm diameter; bolt with nut (M12)

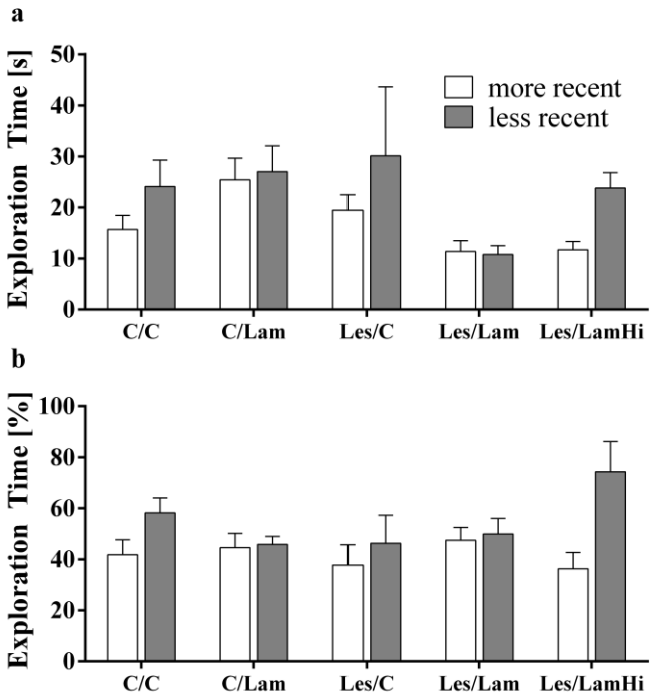

Fig 4.6: Results of the object recency test in absolute (a) and percentual (b) representation. In most groups the animals spent slightly more time exploring the object they encountered less recently, but the difference was only significant within the Les/LamHi group (P = 0.008).

Since the test in its current form does not fulfill the control condition, a significantly reduced exploration time spent on the object encountered more recently, it is not suitable for the investigation of forebrain defects.

4.2.3 Attentional set shift task

The attentional set shift task, a rather complex experiment that assesses not only working memory, but also cognitive flexibility, has been introduced in the scope of a bachelor thesis. The method was modified from an earlier study which demonstrated disturbances caused by lesions of the mPFC in a task that requires repeated shifting from one sensory dimension to another (Birrell and Brown 2000).

The experiment was conducted in a box (71 x 30.5 x 30.5 cm) with three compartments: The start compartment (1/3 of the box) was separated from the test compartment by a guillotine door. Opposite to the guillotine door two small plant pots (height: 6 cm, inner diameter at top: 7.5 cm) were placed, separated by a plexiglass wall. The animals' task

was to find a food reward in the plant pots based on either the digging material[3] in the pots or an artificial scent[4], while being challenged by a repeated shift of the relevant stimulus.

After being habituated to the apparatus and the plant pots, the rats were trained to dig for a food reward, a piece of a breakfast cereal (Choko-Chips; Gut & Günstig; Edeka Germany) in one of the plant pots. Both plant pots were baited and filled with standard cage litter as digging material. Each training session consisted of ten runs, a run was counted a success, if the animal found its reward within 60 seconds. The training was completed when the rat succeeded on at least 70 % of the runs for three consecutive days.

During the test phase just one of the pots was baited. The rat had to learn which stimulus to associate with the reward and adapt the strategy several times to the changed conditions during the test phase. The test phase consisted of nine stages each of which was completed once the rat found the bait in seven consecutive runs.

(1) Simple discrimination: One stimulus dimension is introduced to mark the baited pot (e.g. strawberry scent marks the baited pot, cinnamon scent the unbaited one). Meanwhile the other stimulus dimension is kept constant (e.g. wood shavings in both pots).

(2) Compound discrimination: A second, irrelevant stimulus is added. E.g.: The baited pot is still marked by strawberry scent but now the pots are randomly filled with wood shavings or konfetti.

(3) Reversal of compound discrimination (CDrev): The baited pot is still marked by the same stimulus dimension, but the stimulus that formerly marked the unbaited pot now marks the baited one (e.g. now cinnamon scent leads to the reward, strawberry scent is applied to the unbaited pot).

(4) Repetition of CDrev: A repetition of (3) to ensure the rat has memorized the new strategy the next day.

(5) Intradimensional shift: Digging material and scent are both replaced but the relevant stimulus dimension is kept constant (e.g. a rat trained to find the reward first by straw-

3 Available digging materials: wood shavings, cherry stones, cotton wool, straw chippings, wool, confetti, cage litter, clay granulate

4 Available scents: strawberry, cinnamon, vanilla, aniseed, carnation, jasmine, thyme, citrus (all: Dragonspice Naturwaren, Germany)

berry scent, then by cinnamon scent, now has to chose the pot marked by thyme scent, not by vanilla scent. The digging material is still irrelevant for bait finding).
(6) Reversal of intradimensional shift: The stimuli marking baited and unbaited pot are reversed. The other stimulus dimension remains irrelevant. (Example: Now vanilla scent marks the baited pot, the thyme scented one is unbaited.)
(7) Repetition of the intradimensional shifting: A repetition of (6) to ensure the rat has memorized the new strategy the next day.
(8) Extradimensional shift: Both the scents and the digging materials are exchanged and the relevant and irrelevant stimulus dimensions are reversed (e.g. a rat that had to find the bait based on the scent now has to pay attention to the digging material. The food reward is placed in the pot filled with cotton wool, not the one with cherry stones. The scents, aniseed and carnation, are irrelevant).
(9) Reversal of extradimensional shift: The relevant stimulus dimension remains identical to (8) but the stimuli marking baited and unbaited pot are reversed. (Example: Now the food reward is hidden in the pot filled with cherry stones, not the one containing cotton wool. The scents remain irrelevant.)

As measures of performance the number of errors, the number of trials as well as the time until the criterion was fulfilled were assessed. Within the bachelor project seven sham-lesioned and laminin-treated rats, as well as five lesioned and laminin-treated rats were tested. Although some differences between the treatment groups were found, the experiments revealed that the true reason for these differences rather lies in an inherent problem of the method applied.
The experimental design assumes that a discrimination based on scent can be learned equally fast as a discrimination based on digging material. To test this assumption the sham-lesioned group was subdivided based on which stimulus dimension was relevant first. It turned out that these sub-groups differed significantly from each other (two-way repeated measures ANOVA: $P = 0.033$; Fig 4.7). Under the given experimental conditions the discrimination based on the digging material required markedly less trials compared to a scent-based discrimination. This phenomenon was not only present at the start of the experiment where the animals needed more trials to succeed in the simple discrimination and the compound discrimination (post hoc Tukey test: phase (1):

$P < 0.001$; phase (2): $P = 0.002$; phase 3: $P < 0.001$), also the extradimensional shift revealed that the rats that now had to focus on scent needed significantly more trials to succeed (phase 8 and 9: $P < 0.001$).

Taken together, the experimental outcome is largely influenced by the group assignments: If one treatment group included more animals that start with a given stimulus dimension, the results may be skewed. As a simple workaround all animals could start with the identical stimulus dimension. However, in this case the extradimensional shift might be represented unrealistically easy or difficult due to the selected stimulus order. Ideally, both discrimination tasks should be fulfilled at an equal level of difficulty. It is possible that the chosen amount of scent was to high so that the animals could not locate its origin within the box. Alternatively, the stimulus dimension scent could be replaced for example by sound. Two speakers could be placed in the vicinity of the plant pots and play different tones. However, further experiments would be needed to verify if this stimulus dimension is similarly easy to discriminate for rats as the digging material.

The attentional set shift task offers high value in the assessment of forebrain functions. In contrast to other tests it not only requires an intact working memory, but also challenges other executive functions such as inhibitory control and cognitive flexibility. An earlier study demonstrated severe impairments of rats with a lesioned mPFC after the extradimensional shift (Birrell and Brown 2000). On the other hand, the experimental procedure would have required further refinement to match the demand of both stimulus dimensions. Furthermore, the complex experimental procedure turned out to be especially time consuming compared to other behavioral tests, resulting in a low throughput of animals. Therefore, and with respect to the histological results the attentional set shift task was not further pursued.

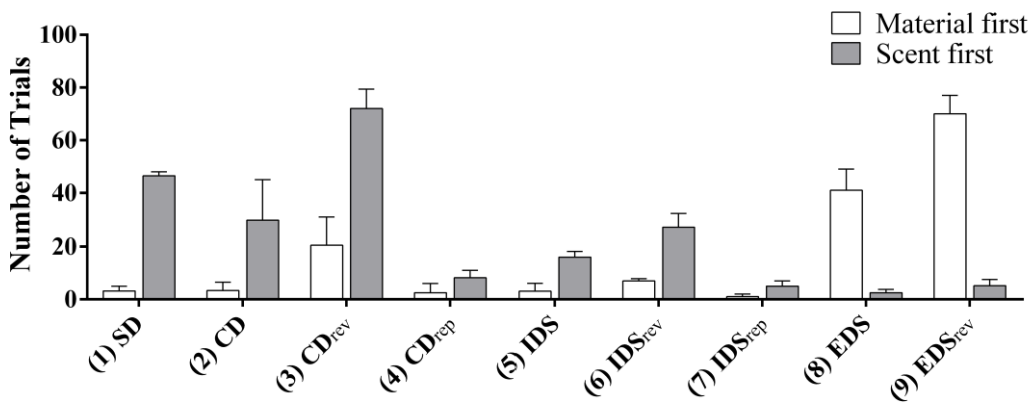

Fig 4.7: Performance of the control group in the attentional set shift task. Isolated analysis of the control group reveals the inherent problem with the experimental procedure: Animals that first have to discriminate based on the scent needed significantly more trials at the start of the experiment. Likewise, the animals that were required to perform a scent-based discrimination in phase 8 and 9 needed more trials to complete the task.

4.2.4 Open field test

The open field test was conducted in an Actimot apparatus (TSE Germany), which consisted of three infrared-beam monitored acrylic boxes (48 x 48 x 48 cm; 16 infrared light barriers at ground level and 16 infrared light barriers to detect rearings), visually shielded to three sides. The rats were allowed to freely move within the boxes for 30 minutes while their momentary position within the box as well as the rearings were constantly recorded based on the infrared light barrier data. Analyzed parameters were the activity [% of time], the distance traveled [m], the number of rearings, and the time spent at the center of the box [s]. The analysis was performed on five minute intervals.
As expected, the activity, distance traveled, and the number of rearings decreased over the 30 minutes. This was observed independent of the treatment (data not shown). For the time spent at the center, this decrease was less pronounced and the Les/Lam group showed significantly more rearings in the 10-15 minute interval than all other treatment groups (two-way repeated measures ANOVA; both: $P \leq 0.001$).
The data was also analyzed as sum over the 30 minutes, which also allows for a more compact presentation of the data (Fig 4.8). This cumulative data analysis revealed no significant group effects (one-way ANOVA for each parameter; factor: treatment).

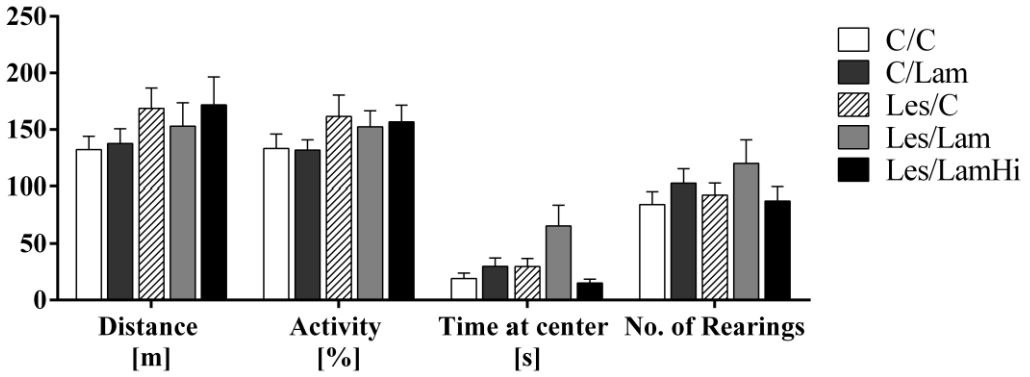

Fig 4.8: Summarized activity of the animals in the open field test. Data is presented as sums of the five minute intervals over the total duration of the test (30 min). Significant differences between the treatment groups were found for the time spent at the center and number of rearings, but only for single five minute intervals (see text). The cumulative analysis revealed no significant treatment effects.

4.2.5 Conclusion

The behavioral experiments were included into the original study design under the premise that the successful relocation of neuroblasts from the RMS into a prefrontal forebrain lesion is succeeded by a reinnervation of the damaged tissue. Histological examination of the first batches of lesioned and laminin-treated rats revealed that, at least in the given amount of time, no such reinnervation occurs. Although the presence (Dixon et al. 2015) or proliferation (Li et al. 2010) of neuronal precursor cells has been associated with less severe behavioral symptoms following TBI or ischemia, respectively, it seems unlikely that a relatively low number of neuroblasts can compensate for the large scale loss caused by the ibotenic acid induced lesions. Furthermore, the results of the behavioral experiments were rather unclear. The attentional set shift task would have required a revision of the methodology to allow interpretation of the results. The T-Maze alternation task and the object recency task failed to reliably find a lesion effect which would be a prerequisite to assess the possibly beneficial effects of a relocation of neuroblasts into the lesion. It remains unclear if such an effect could be found by acquisition of a larger sample size.

Another reason for the unclear results of the behavioral tests might lie in the variation in the location and extent of the brain lesions achieved in this study (cp. 4.1.3). Earlier

studies found different effects of lesions of the prefrontal cortex on the performance of a maze-based working memory task dependent on the subregion affected (Seamans et al. 1995; Sánchez-Santed et al. 1997). Other studies found that the behavioral effects of prefrontal cortex lesions are dependent on lesion size (de Bruin et al. 1994), or the hemisphere affected (Sullivan and Gratton 2002). Therefore, in follow up projects opted for behavioral experiments, the lesioning method should be refined to obtain more predictable brain damage.

4.3 Cell proliferation marker

The original study design included the application of bromdesoxyuridin (BrdU) as a cell proliferation marker. BrdU is an analog to the nucleoside thymidine and is incorporated into the DNA during the S-phase of the cell cycle. It can be detected immunohistochemically and therefore allows identification of cells that were generated while the substance was present (Taupin 2007).

In the scope of the preliminary experiments no reliable BrdU-staining could be achieved.

The animals that underwent the behavioral tests and MRI scans received subcutaneous BrdU injections (60 mg / kg bodyweight) at three consecutive days after the second surgery.

Although in some cases cells in the RMS of BrdU treated animals were detectable (Fig 4.9), the staining showed relatively low contrast against the background and the overall staining quality varied vastly between subjects. Numerous attempts to improve the histological method (higher primary and secondary antibody concentrations, different incubation times, variations of the hydrochloric acid based antigen retrieval methods) have not led to clearer results. Other possible sources of error lie in the BrdU-treatment itself. The dose has been kept comparably low (Wojtowicz and Kee 2006) in order to avoid severe side effects of the mutagenic substance which is especially critical due to the long survival time after the surgeries (Taupin 2007). Moreover, with single BrdU injections timing is critical. As discussed in 2.5.3 the BrdU has to be available systemically when the cells that are later redirected into the brain lesion proliferate. The BrdU injections were timed based on literature on physiological neuroblast migration speed from the SVZ towards the OB (Peretto et al. 1997; Brown et al. 2003). However, if the migration

speed throughout the RMS is non-linear, this estimation might be incorrect thus reducing the number of marked neuroblasts within the lesion: If the neuroblasts in the lesion were generated before or after the BrdU treatment, they would not have been detectable by this method. Furthermore, additional histological measures are necessary to identify the type of BrdU-positive cells, since the lesion causes increased proliferation of glia cells (Liu et al. 2000).

The final study design opted for the application of DCX, a marker for immature neurons. It can be detected immunohistologically, without the *in vivo* application of additional substances. On the other hand, this method does not provide insight into the time of birth of the detected cells, and therefore does not prove that the detected cells in the lesions of laminin or endo-N treated animals were indeed of postnatal origin. Therefore, the combination of DCX immunohistochemistry with BrdU, applied at different times, would be a useful follow up to the studies presented in this thesis. Alternatively, the single cell MRI marking discussed above (see 4.1.6), combined with a *post mortem* DCX detection could potentially provide similar insights with a lower number of animals necessary.

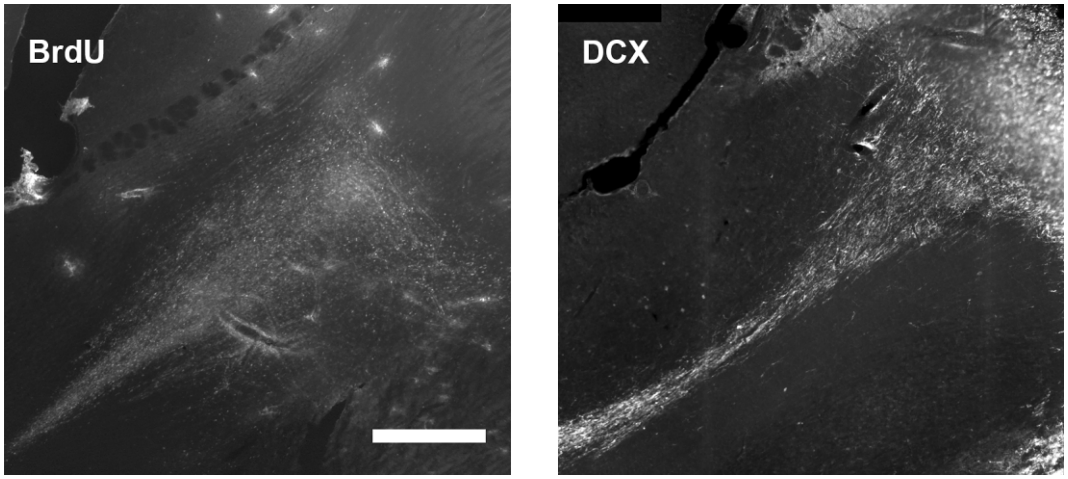

Fig 4.9: Example of a successful BrdU staining in comparison to a DCX staining. Both images show a sagittal section of the rostral end of the RMS. Scale bar: 500 µm; caudal: left; dorsal: up.

5 Résumé and outlook

5.1 Conclusion

Within the scope of this thesis two methods were applied that enabled the redirection of physiologically generated neuroblasts into forebrain lesions of adult rats.

The injection of a narrow tract of laminin, reaching from the tissue dorsal to the neuroblasts' migration path, the RMS, towards the brain lesion routed the neuroblasts towards the damaged tissue. Once the cells had reached the end of the laminin tract, they continued migrating and spread within the lesion. Administration of a higher concentrated laminin tract did not increase the density of relocated neuroblasts but led to an enhanced reliability of the method. However, only single cells showed the morphological and immunohistochemical signs of differentiation into a neuronal cell type.

The intraventricular injection of the enzyme endoN inhibits the formation of cell chains, which is usually observed for SVZ-derived neuroblasts. This is not only known to decrease the speed of cell migration in the RMS but also allows the dispersion of cells from the otherwise rigid migration path (Ono et al. 1994). Application of the enzyme to adult rats with forebrain lesions resulted in migration of neuroblasts into the damaged tissue. In this study no signs of neuroblast differentiation were found.

Consequently, hypotheses (1) and (2) (cp. 1.2.3) are validated: Both methods caused neuroblasts to leave the RMS and migrate towards the lesion. The finding that the cells disperse within the lesion but not into other brain regions indicates that neuroblasts are further guided by lesion induced migration cues.

However, hypothesis (3) can not be validated by the data at hand. Only in the laminin study single cells, expressing both the neuronal precursor marker DCX and the marker for adult neurons, NeuN, were found. The experiments conducted in this study do not provide further insight into the fate of relocated neuroblasts after they reach the lesioned tissue. There are multiple possible paths for the neuroblasts: The cells could stay in their immature state and accumulate within the lesion or die there. Then again, it is conceivable that the cells differentiate into a glial cell type (Reynolds and Weiss 1992). Furthermore, it is possible that some neuroblasts differentiated into neurons, but were not detectable with the analysis applied. The markers DCX and NeuN are only expressed si-

multaneously for a short time (Brown et al. 2003) and once the neuroblast has differentiated, it is virtually indistinguishable from the surrounding neurons (Gage 2000). To further investigate the fate of the redirected neuroblasts the application of a cell marking technique, such as a proliferation marker or a retrovirus (Mozdziak and Schultz 2000), is essential. Furthermore, this would allow for follow-up experiments that assess the feasibility of *in vivo* fate determination by administration of various growth factors, substances that have been demonstrated effective in *in vitro* studies before (Reynolds and Weiss 1992; Ahmed et al. 1995; Memberg and Hall 1995; Arsenijevic and Weiss 1998).
The originally planned MRI-based longitudinal observation did not meet expectations. Although the lesions were clearly detectable in the conducted scans, the minute changes due to relocated neuroblasts could not be characterized. Only if the presented treatments are extended in a way that leads to a substantial reinnervation of the brain lesions, the DTI method might provide insight into the time scale of these processes.
However, different MRI methods could be applied to further investigate the redirection of RMS-neuroblasts. Especially the labeling of neuroblasts with iron oxide nanoparticles could provide insight into the physiological and manipulated migration of these cells. However, the selective cell targeting, as well as potential neurotoxic side effects yield new challenges.

5.2 Perspective: The role of neuroblast redirection in future treatment of human brain damage

The lack of a treatment option for structural brain damage in humans was the main motivation for this project. An optimal clinical treatment of brain damage would probably be threefold: Occurrence of brain lesions, caused by neurodegenerative diseases or secondary effects of acute brain injury should be prevented in the first place. As pointed out in 1.1.2 the death of neurons in the CNS is a highly regulated process and therefore offers a plethora of potential interventions. However, so far no effective neuroprotective agent has been identified (Stocchetti et al. 2015).
The second promising approach to treatment of structural brain damage is based on neuroplasticity. The brains innate ability to shift tasks from injured tissue to other brain areas is clinically supported by rehabilitation measures predominantly to regain motor skills (Dimyan and Cohen 2011; Nudo 2013), but also improve sensory and cognitive

deficits (Kleim and Jones 2008). Similarly, animal models of neurodegenerative diseases (Laviola et al. 2008) and TBI (Bondi et al. 2014) have demonstrated the beneficial effects of environmental enrichment. Additionally, novel methods such as electrically stimulating brain-computer interfaces or pharmacological intervention could further enhance neuroplasticity (Dimyan and Cohen 2011).

The introduction of neuronal precursor cells into an area of brain damage, either by grafting of *in vitro* cultured stem cells or, as presented here, by relocation of endogenous neuroblasts, is a promising approach for a future treatment of brain damage. The newly introduced cells could take over the function of the lost ones and could potentially facilitate re-learning of lost abilities. However, although the idea of direct replacement of lost neurons is appealing, it raises a number of questions: Will the new cells survive in the lesion environment? If so, will they differentiate into a functionally relevant type of neuron? Will these cells functionally interact and integrate into the surrounding tissue? What can be done to support a neuronal precursor cell to become a useful brain cell, integrated in a functional network?

Recently, adult neurogenesis in humans has become a topic of debate again: New studies suggest that neurogenesis in humans sharply declines in the early years of life (Sorrells et al. 2018). Although this finding is questioned by others (Snyder 2018; Boldrini et al. 2018), it remains uncertain if the methods of relocation of SVZ neuroblasts will ever become relevant in a clinical application for humans. Nonetheless, in order to answer some of the questions raised above, extensive research on the topic of neuronal replacement is necessary. Since the fate of neuronal precursor cells is largely effected by their microenvironment (Soen et al. 2006), these cells, at some point have to be studied within the context of the living brain. The methods presented in this thesis therefore not only have the potential to directly help finding a cure for structural brain damage, they also provide a sound model with relatively low methodological demands for future research on neuroblasts within brain lesions.

6 Summaries

6.1 English summary

Up until today physical brain damage is incurable. Whenever cells of the central nervous system are lost, either due to a traumatic brain injury, stroke, or neurodegenerative diseases, they are considered irretrievably lost. Nonetheless, the adult mammalian brain features two distinct areas capable of continuous production of neuronal precursor cells: The subgranular zone of the hippocampus and the subventricular zone around the walls of the lateral ventricles. The subventricular zone-derived neuroblasts follow a rigid migratory path, the rostral migratory stream (RMS), through the forebrain towards the olfactory bulb. En route they form elongated aggregates, so called migration chains, which accelerate the migration, but also hinder the cells from leaving the RMS. Within the present project two methods were evaluated with regard to their potential to redirect neuroblasts from the RMS of adult rats towards a nearby brain lesion.

Study 1:

In a first stereotactic surgery ibotenic acid was infused into the medial prefrontal cortex of adult rats, which caused an excitotoxic lesion of the tissue. In a second surgery five days later, a tract of a laminin solution was injected, reaching barely from the dorsal border of the RMS to the lesion. Due to the known chemoattractive properties of laminin single neuroblasts left the RMS and followed the injection tract towards the lesion. Here the cells dispersed, probably following other migratory cues, in random directions. A higher dose of laminin did not increase the number of relocated cells, but enhanced the reliability of the method.
In a few of these cells markers for neuroblasts as well as adult neurons could be detected. Together with the morphological properties of these cells, this serves as an indicator for the differentiation towards a neuronal cell type.

Study 2:

Like in the first study, the rats first received excitotoxic lesions of the medial prefrontal cortex. Five days later the enzyme endoN was infused into the ventricle of the animals.

As known from previous studies, endoN cleaves the polysialic acid moiety from the neural cell adhesion molecule. This results in break up of the migratory chains and is followed by dispersion of neuroblasts from the RMS and a widened morphology of the RMS.

These findings could be reproduced, despite the fact that the polysialisated neural cell adhesion molecule could still be detected immunohistologically in endoN-treated animals. Thus it is possible, that the effect of the enzyme has worn off within the survival time of three weeks.

In the previously lesioned and endoN-treated animals a migration of neuroblasts from the RMS towards the lesioned tissue was observed. Furthermore, the cells dispersed within the lesion site. However, in this study no signs of neuronal differentiation were found. A higher dose of the enzyme had no impact on the number of relocated cells.

Further experiments

Additionally, magnetic resonance imaging and behavioral methods that were not included in the publications are presented and discussed in this thesis.

Originally, the temporal course of the neuroblast relocation and a potential reinnervation of the lesioned tissue should have been characterized by means of diffusion tensor imaging. The method allows insight into the gross amount and anisotropy of water diffusion within the examined tissue and is clinically applied to diagnose neurodegeneration. However, in the context of this project no useful data could be acquired. First, the ibotenic acid-induced lesions varied in their physical extent, which massively complicated the statistical group analysis. Second, the number of relocated neuroblasts was lower than originally expected and an extensive reinnervation was not observed. This would have been a prerequisite for a detectable change in diffusivity.

The behavioral tests resulted in similarly unclear results. In one of the procedures (the attentional set shift task) a systematic error could be determined. Despite the present literature, the other experiments (T-maze alternation task, object recency task, open field) did not yield a reliably lesion-effect. Presumably, this is also caused by the variation of lesion extent, since different subdivisions of the medial prefrontal cortex are involved in different functions.

Conclusion

The studies have been conducted with the perspective of a future development of a clinical treatment of structural brain damage. Due to the fact that neither of the methods led to a reinnervation of the forebrain lesion further studies on the survival and differentiation of the relocated neuroblasts are necessary. Both methods presented in this thesis may serve as sound animal models for this future work.

6.2 German summary

Bis heute gelten physische Hirnschäden als unheilbar. Sind Zellen des Zentralnervensystems gestorben, entweder durch eine traumatische Hirnverletzung, einen Schlaganfall, oder infolge einer neurodegenerativen Erkrankung, gelten diese als unwiederbringlich verloren. Gleichwohl enthält das adulte Säugetierhirn zwei Areale, die fortwährend neuronale Vorläuferzellen bilden: Die subgranuläre Zone des Hippocampus und die subventrikuläre Zone entlang der Wände der lateralen Ventrikel. Die Neuroblasten der subventrikulären Zone folgen einem rigiden Migrationspfad, dem rostralen migratorischen Strom (RMS), durch das Vorderhirn zum olfaktorischen Bulbus. Hierbei bilden die Zellen elongierte Aggregate, sogenannte Migrationsketten, wodurch die Zellwanderung beschleunigt, aber auch ein Auswandern einzelner Zellen aus dem Migrationspfad erschwert wird. Im vorliegenden Projekt wurden zwei Verfahren evaluiert um die Neuroblasten aus dem RMS in eine benachbarte Hirnläsion umzuleiten.

Studie 1:

In einer ersten stereotaktischen Operation wurde Ibotensäure in den medialen präfrontalen Cortex adulter Ratten infundiert, was zu einer exzitotoxischen Läsion des Gewebes führte. In einer zweiten Operation fünf Tage später wurde dann ein Trakt einer Lamininlösung injiziert, der knapp dorsal des RMS beginnt und bis in das läsionierte Gewebe reichte. Aufgrund der bekannten chemoattraktiven Wirkung des Laminins wanderten daraufhin einzelne Zellen aus dem RMS aus und folgen dem Injektionstrakt bis in die Läsion. Hier verteilten sich die Zellen, anscheinend anderen Migrationssignalen folgend, in unterschiedliche Richtungen. Durch eine höhere Dosis des Laminins konnte die mittlere Anzahl der umgeleiteten Zellen nicht erhöht werden, die Methode funktionierte jedoch zuverlässiger.

In einzelnen dieser Zellen konnten sowohl Marker für Neuroblasten als auch für adulte Neurone nachgewiesen werden. Zusammen mit den morphologischen Merkmalen dieser Zellen dient dies als Hinweis auf eine Differenzierung zu einem neuronalen Zelltyp hin.

Studie 2:

Auch in dieser Studie wurden zunächst, wie oben beschrieben, exzitotoxische Läsionen des medialen präfrontalen Cortex adulter Ratten hervorgerufen. Fünf tage später wurde das Enzym endoN in den Ventrikel der Tiere infundiert.
Aus vorherigen Studien ist bekannt, dass endoN Polysialinsäure vom neuralen Zelladhäsionsmolekül abspaltet. Dadurch werden die Migrationsketten aufgespalten, was zu einem Ausstrom von Neuroblasten aus dem RMS und einer verbreiterten Morphologie des RMS führt. Diese Ergebnisse konnten reproduziert werden, obwohl auch in endoN-behandelten Tieren noch das polysialinierte neurale Zelladhäsionsmolekül immunhistologisch nachgewiesen werden konnte. Es ist folglich möglich, dass die Wirkung des Enzyms nach der Überlebenszeit von drei Wochen wieder nachgelassen hat.
In den zuvor läsionierten und endoN behandelten Tieren konnte eine Migration von Neuroblasten vom RMS zum geschädigten Gewebe beobachtet werden. Außerdem verteilten sich die Zellen innerhalb der Hirnläsion. In dieser Studie wurden keine Hinweise auf die Differenzierung der Neuroblasten zu Neuronen gefunden. Für zwei verschiedene Dosierungen des Enzyms konnten keine Unterschiede in der Anzahl von Zellen in der Läsion festgestellt werden.

Weitere Experimente

Im weiteren Verlauf dieser Arbeit werden magnetresonanztomographische und verhaltensbiologische Methoden vorgestellt und diskutiert, die nicht in die Veröffentlichungen eingeflossen sind.
Mittels Diffusionstensor Bildgebung sollte ursprünglich der zeitliche Verlauf der Einwanderung von Neuroblasten und eine mögliche Wiederherstellung des läsionierten Gewebes charakterisiert werden. Die Methode erlaubt Rückschlüsse auf den Grad und die Anisotropie der Wasserdiffusion im untersuchten Gewebe und wird klinisch zur Diagnose von Neurodegeneration eingesetzt. Im Kontext der vorliegenden Studien konnten jedoch keine aussagekräftigen Ergebnisse erzielt werden. Zum einen variierten die mittels

Ibotensäure hervorgerufenen Läsionen in ihrer physischen Ausdehnung, was eine statistische Auswertung der Behandlungsgruppen massiv erschwerte. Zum anderen war die Anzahl von umgeleiteten Neuroblasten geringer als ursprünglich angenommen und eine großflächige Reinnervation des läsionierten Gewebes blieb aus, was eine Voraussetzung für nachweisbare Änderungen der Diffusivität gewesen wäre.
Auch die Verhaltensexperimente lieferten keine klaren Ergebnisse. In einer experimentellen Methode (*Attentional set shift task*) konnte ein systematische Fehler nachgewiesen werden, in den anderen Versuchen (*T-maze alternation task, Object recency task, Open field*) konnte entgegen der vorhandenen Literatur kein stabiler Läsionseffekt nachgewiesen werden. Vermutlich ist dies ebenfalls in der Variation der Läsionsausdehnung begründet, da unterschiedliche Untereinheiten des medialen präfrontalen Cortex mit verschiedenen Funktionen assoziiert werden.

Fazit

Die Studien wurden vor dem Hintergrund der Entwicklung einer klinischen Behandlungsmöglichkeit struktureller Hirnschäden durchgeführt. Da die untersuchten Methoden allerdings keine Reinnervation der Läsion bewirken, sind weitere Untersuchungen hinsichtlich des Überlebens und der Differenzierung der umgeleiteten Neuroblasten nötig. Beide Studien können hierfür solide Tiermodelle liefern.

7 References

Aarsland D, Andersen K, Larsen JP, et al (2001) Risk of dementia in Parkinson's disease: a community-based, prospective study. Neurology. doi: 10.1212/WNL.56.6.730

Ahmed S, Reynolds B, Weiss S (1995) BDNF enhances the differentiation but not the survival of CNS stem cell- derived neuronal precursors. J Neurosci 15:5765–5778. doi: 10.1523/JNEUROSCI.15-08-05765.1995

Albanese BJ, Macatee RJ, Stentz LA, et al (2018) Interactive effects of cumulative lifetime traumatic brain injuries and combat exposure on posttraumatic stress among deployed military personnel. Cogn Behav Ther 1–12. doi: 10.1080/16506073.2018.1478446

Alexander AL, Lee JE, Lazar M, Field AS (2007) Diffusion tensor imaging of the brain. Neurotherapeutics 4:316–329. doi: 10.1016/j.nurt.2007.05.011

Altman J (1969) Autoradiographic and histological studies of postnatal neurogenesis. IV. Cell proliferation and migration in the anterior forebrain, with special reference to persisting neurogenesis in the olfactory bulb. J Comp Neurol 137:433–457. doi: 10.1002/cne.901370404

Altman J, Das GD (1965) Post-natal origin of microneurones in the rat brain. Nature 207:953

Andrade N, Komnenovic V, Blake SM, et al (2007) ApoER2/VLDL receptor and Dab1 in the rostral migratory stream function in postnatal neuronal migration independently of Reelin. Proc Natl Acad Sci U S A 104:8508–13. doi: 10.1073/pnas.0611391104

Andreas K, Georgieva R, Ladwig M, et al (2012) Highly efficient magnetic stem cell labeling with citrate-coated superparamagnetic iron oxide nanoparticles for MRI tracking. Biomaterials 33:4515–4525. doi: 10.1016/J.BIOMATERIALS.2012.02.064

Andriessen TMJC, Jacobs B, Vos PE (2010) Clinical characteristics and pathophysiological mechanisms of focal and diffuse traumatic brain injury. J Cell Mol Med 14:2381–2392. doi: doi:10.1111/j.1582-4934.2010.01164.x

Arsenijevic Y, Weiss S (1998) Insulin-Like Growth Factor-I Is a Differentiation Factor for Postmitotic CNS Stem Cell-Derived Neuronal Precursors: Distinct Actions from Those of Brain-Derived Neurotrophic Factor. J Neurosci 18:2118–2128. doi: 10.1523/JNEUROSCI.18-06-02118.1998

Arvidsson A, Collin T, Kirik D, et al (2002) Neuronal replacement from endogenous precursors in the adult brain after stroke. Nat Med 8:963–970

Bano D, Nicotera P (2007) Ca^{2+} Signals and Neuronal Death in Brain Ischemia. Stroke 38:674–676. doi: 10.1161/01.STR.0000256294.46009.29

Barres BA, Jacobson MD, Schmid R, et al (1993) Does oligodendrocyte survival depend on axons? Curr Biol 3:489–497. doi: 10.1016/0960-9822(93)90039-Q

Bartus RT (2000) On Neurodegenerative Diseases, Models, and Treatment Strategies: Lessons Learned and Lessons Forgotten a Generation Following the Cholinergic Hypothesis. Exp Neurol 163:495–529. doi: 10.1006/exnr.2000.7397

Basser PJ, Mattiello J, LeBihan D (1994) MR diffusion tensor spectroscopy and imaging. Biophys J 66:259–267. doi: 10.1016/s0006-3495(94)80775-1

Basser PJ, Pierpaoli C (1996) Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI. J Magn Reson 213:560–570. doi: 10.1016/j.jmr.2011.09.022

Bates GP, Dorsey R, Gusella JF, et al (2015) Huntington disease. Nat Rev Dis Prim 1:15005. doi: 10.1038/nrdp.2015.5

Battista D, Rutishauser U (2010) Removal of Polysialic Acid Triggers Dispersion of Subventricularly Derived Neuroblasts into Surrounding CNS Tissues. J Neurosci 30:3995–4003. doi: 10.1523/JNEUROSCI.4382-09.2010

Beaulieu C (2002) The basis of anisotropic water diffusion in the nervous system - a technical review. NMR Biomed 15:435–455. doi: 10.1002/nbm.782

Becker CG, Artola a, Gerardy-Schahn R, et al (1996) The polysialic acid modification of the neural cell adhesion molecule is involved in spatial learning and hippocampal long-term potentiation. J Neurosci Res 45:143–52. doi: 10.1002/(SICI)1097-4547(19960715)45:2<143::AID-JNR6>3.0.CO;2-A

Belmadani A (2006) Chemokines Regulate the Migration of Neural Progenitors to Sites of Neuroinflammation. J Neurosci 26:3182–3191. doi: 10.1523/JNEUROSCI.0156-06.2006

Belvindrah R, Hankel S, Walker J, et al (2007) Beta1 integrins control the formation of cell chains in the adult rostral migratory stream. J Neurosci 27:2704–17. doi: 10.1523/JNEUROSCI.2991-06.2007

Bergmann O, Liebl J, Bernard S, et al (2012) The Age of Olfactory Bulb Neurons in Humans. Neuron 74:634–639. doi: 10.1016/j.neuron.2012.03.030

Birrell JM, Brown VJ (2000) Medial frontal cortex mediates perceptual attentional set shifting in the rat. J Neurosci 20:4320–4. doi: 10.1523/JNEUROSCI.20-11-04320.2000

Boldrini M, Fulmore CA, Tartt AN, et al (2018) Human Hippocampal Neurogenesis Persists throughout Aging. Cell Stem Cell 22:589–599.e5. doi: 10.1016/j.stem.2018.03.015

Bondi CO, Klitsch KC, Leary JB, Kline AE (2014) Environmental enrichment as a viable neurorehabilitation strategy for experimental traumatic brain injury. J Neurotrauma 31:873–88. doi: 10.1089/neu.2014.3328

Bonfanti L (2013) The (real) neurogenic/gliogenic potential of the postnatal and adult brain parenchyma. ISRN Neurosci 2013:354136. doi: 10.1155/2013/354136

Brown JP, Couillard-Després S, Cooper-Kuhn CM, et al (2003) Transient Expression of Doublecortin during Adult Neurogenesis. J Comp Neurol 467:1–10. doi: 10.1002/cne.10874

Burgess A, Wainwright SR, Shihabuddin LS, et al (2008) Polysialic acid regulates the clustering, migration, and neuronal differentiation of progenitor cells in the adult hippocampus. Dev Neurobiol 68:1580–1590. doi: 10.1002/dneu.20681

Burns A, Iliffe S (2009) Dementia. BMJ 338:b75. doi: 10.1136/BMJ.B75

Burstyn-Cohen T, Tzarfaty V, Frumkin A, et al (1999) F-Spondin is required for accurate pathfinding of commissural axons at the floor plate. Neuron 23:233–46. doi: 10.1016/S0896-6273(00)80776-X

Chazal G, Durbec P, Jankovski A, et al (2000) Consequences of neural cell adhesion molecule deficiency on cell migration in the rostral migratory stream of the mouse. J Neurosci 20:1446–57. doi: 10.1523/JNEUROSCI.20-04-01446.2000

Cheng MY, Leslie FM, Zhou Q-Y (2006) Expression of prokineticins and their receptors in the adult mouse brain. J Comp Neurol 498:796–809. doi: 10.1002/cne.21087

Chiang T, Messing RO, Chou W-H (2011) Mouse model of middle cerebral artery occlusion. J Vis Exp. doi: 10.3791/2761

Chiaramello S, Dalmasso G, Bezin L, et al (2007) BDNF/ TrkB interaction regulates migration of SVZ precursor cells via PI3-K and MAP-K signalling pathways. Eur J Neurosci 26:1780–1790. doi: 10.1111/j.1460-9568.2007.05818.x

Choi DW (1987) Ionic dependence of glutamate neurotoxicity. J Neurosci 7:369–79. doi: 10.1523/JNEUROSCI.07-02-00369.1987

Clarke CE (2007) Parkinson's disease. BMJ 335:441–5. doi: 10.1136/bmj.39289.437454.AD

Committee on Gulf War and Health (2005) Gulf War and Health: Long-Term Effects of Blast Exposures. Paperback 9:1–5. doi: 10.7205/MILMED-D-17-00397

Connolly BS, Lang AE (2014) Pharmacological treatment of parkinson disease: A review. JAMA 311:1670–1683. doi: 10.1001/jama.2014.3654

Conover JC, Doetsch F, Garcia-Verdugo J-M, et al (2000) Disruption of Eph/ephrin signaling affects migration and proliferation in the adult subventricular zone. Nat Neurosci 3:1091–1097. doi: 10.1038/80606

Courtès S, Vernerey J, Pujadas L, et al (2011) Reelin controls progenitor cell migration in the healthy and pathological adult mouse brain. PLoS One 6:e20430. doi: 10.1371/journal.pone.0020430

Cummings J (2017) Disease modification and Neuroprotection in neurodegenerative disorders. Transl Neurodegener 6:25. doi: 10.1186/s40035-017-0096-2

Curtis MA, Kam M, Faull RLM (2011) Neurogenesis in humans. Eur J Neurosci 33:1170–1174. doi: 10.1111/j.1460-9568.2011.07616.x

Curtis MA, Kam M, Nannmark U, et al (2007) Human Neuroblasts Migrate to the Olfactory Bulb via a Lateral Ventricular Extension. Science (80-) 315:1243–1249. doi: 10.1126/science.1136281

David LS, Schachner M, Saghatelyan A (2013) The extracellular matrix glycoprotein tenascin-R affects adult but not developmental neurogenesis in the olfactory bulb. J Neurosci 33:10324–39. doi: 10.1523/JNEUROSCI.5728-12.2013

Dayalu P, Albin RL (2015) Huntington Disease: Pathogenesis and Treatment. Neurol Clin 33:101–114. doi: 10.1016/j.ncl.2014.09.003

de Bruin JPC, Sánchez-Santed F, Heinsbroek RPW, et al (1994) A behavioural analysis of rats with damage to the medial prefrontal cortex using the morris water maze: evidence for behavioural flexibility, but not for impaired spatial navigation. Brain Res 652:323–333. doi: 10.1016/0006-8993(94)90243-7

Deacon RMJ, Rawlins JNP (2006) T-maze alternation in the rodent. Nat Protoc 1:7–12. doi: 10.1038/nprot.2006.2

Decker L, Durbec P, Rougon G, Evercooren AB Van (2002) Loss of polysialic residues accelerates CNS neural precursor differentiation in pathological conditions. Mol Cell Neurosci 19:225–238. doi: 10.1006/mcne.2001.1072

Dimyan MA, Cohen LG (2011) Neuroplasticity in the context of motor rehabilitation after stroke. Nat Rev Neurol 7:76–85. doi: 10.1038/nrneurol.2010.200

Divac I (1971) Frontal lobe system and spatial reversal in the rat. Neuropsychologia 9:175–183. doi: 10.1016/0028-3932(71)90041-8

Dixon KJ, Theus MH, Nelersa CM, et al (2015) Endogenous neural stem/progenitor cells stabilize the cortical microenvironment after traumatic brain injury. J Neurotrauma 32:753–764. doi: 10.1089/neu.2014.3390

Doetsch F, García-Verdugo A, Alvarez-Buylla J (1997) Cellular Composition and Three-Dimensional Organization of the Subventricular Germinal Zone in the Adult Mammalian Brain. J Neurosci 17(13):5046–61. doi: 10.1002/cne.902890106

Dusart I, Marty S, Peschanski M (1991) Glial changes following an excitotoxic lesion in the CNS—II. Astrocytes. Neuroscience 45:541–549. doi: 10.1016/0306-4522(91)90269-T

Eguchi Y, Shimizu S, Tsujimoto Y (1997) Intracellular ATP levels determine cell death fate by apoptosis or necrosis. Cancer Res 57:1835–40

Elmore S (2007) Apoptosis: A Review of Programmed Cell Death. Toxicol. Pathol. 35:495–516

Emsley JG, Hagg T (2003) α6β1 integrin directs migration of neuronal precursors in adult mouse forebrain. Exp Neurol 183:273–285. doi: 10.1016/s0014-4886(03)00209-7

Emsley JG, Mitchell BD, Kempermann G, Macklis JD (2005) Adult neurogenesis and repair of the adult CNS with neural progenitors, precursors, and stem cells. Prog Neurobiol 75:321–341. doi: 10.1016/j.pneurobio.2005.04.002

Ernst A, Alkass K, Bernard S, et al (2014) Neurogenesis in the Striatum of the Adult Human Brain. Cell 156:1072–1083. doi: 10.1016/j.cell.2014.01.044

Fallon J, Reid S, Kinyamu R, et al (2000) In vivo induction of massive proliferation, directed migration, and differentiation of neural cells in the adult mammalian brain. Proc Natl Acad Sci 97:14686–14691. doi: 10.1073/pnas.97.26.14686

Faul M, Coronado V (2015) Chapter 1 - Epidemiology of traumatic brain injury. In: Grafman J, Salazar AM (eds) Handbook of Clinical Neurology. Elsevier, pp 3–13

Finne J (1982) Occurrence of unique polysialosyl carbohydrate units in glycoproteins of developing brain. J Biol Chem 257:11966–11970

Finne J, Finne U, Deagostini-Bazin H, Goridis C (1983) Occurrence of alpha2-8 linked polysialosyl units in a neural cell adhesion molecule. Biochem Biophys Res Commun 112:482–487. doi: 10.1016/0006-291X(83)91490-0

Foster TC (2007) Calcium homeostasis and modulation of synaptic plasticity in the aged brain. Aging Cell 6:319–325. doi: 10.1111/j.1474-9726.2007.00283.x

Friston KJ (Karl J., Ashburner J, Kiebel S, et al (2007) Statistical parametric mapping: the analysis of funtional brain images. Elsevier/Academic Press

Fuchs Y, Steller H (2011) Programmed cell death in animal development and disease. Cell 147:742–58. doi: 10.1016/j.cell.2011.10.033

Gage FH (2000) Mammalian neural stem cells. Science 287:1433–8. doi: 10.1126/SCIENCE.287.5457.1433

Gage FH (2002) Neurogenesis in the adult brain. J Neurosci 22:612–613

Gage FH, Coates PW, Palmer TD, et al (1995) Survival and differentiation of adult neuronal progenitor cells transplanted to the adult brain. Proc Natl Acad Sci USA 92:11879–11883. doi: 10.1073/pnas.92.25.11879

Gallina P, Paganini M, Lombardini L, et al (2010) Human striatal neuroblasts develop and build a striatal-like structure into the brain of Huntington's disease patients after transplantation. Exp Neurol 222:30–41. doi: 10.1016/j.expneurol.2009.12.005

García-Marqués J, De Carlos JA, Greer CA, López-Mascaraque L (2010) Different astroglia permissivity controls the migration of olfactory bulb interneuron precursors. Glia 58:218–230. doi: 10.1002/glia.20918

Gascon E, Vutskits L, Jenny B, et al (2007) PSA-NCAM in postnatally generated immature neurons of the olfactory bulb: a crucial role in regulating p75 expression and cell survival. Development 134:1181–1190. doi: 10.1242/dev.02808

Gebara E, Bonaguidi MA, Beckervordersandforth R, et al (2016) Heterogeneity of Radial Glia-Like Cells in the Adult Hippocampus. Stem Cells 34:997–1010. doi: 10.1002/stem.2266

Ghashghaei HT, Lai C, Anton ES (2007) Neuronal migration in the adult brain: are we there yet? Nat Rev Neurosci 8:141–151. doi: 10.1038/nrn2074

Ghashghaei HT, Weber J, Pevny L, et al (2006) The role of neuregulin-ErbB4 interactions on the proliferation and organization of cells in the subventricular zone. Proc Natl Acad Sci U S A 103:1930–5. doi: 10.1073/pnas.0510410103

Gill J, Mustapic M, Diaz-Arrastia R, et al (2018) Higher exosomal tau, amyloid-beta 42 and IL-10 are associated with mild TBIs and chronic symptoms in military personnel. Brain Inj 1–8. doi: 10.1080/02699052.2018.1471738

Gilley J, Coleman MP (2010) Endogenous Nmnat2 is an essential survival factor for maintenance of healthy axons. PLoS Biol 8:e1000300. doi: 10.1371/journal.pbio.1000300

Glaser T, Brose C, Franceschini I, et al (2007) Neural Cell Adhesion Molecule Polysialylation Enhances the Sensitivity of Embryonic Stem Cell-Derived Neural Precursors to Migration Guidance Cues. Stem Cells 25:3016–3025. doi: 10.1634/stemcells.2007-0218

Gong G, He Y, Concha L, et al (2009) Mapping Anatomical Connectivity Patterns of Human Cerebral Cortex Using In Vivo Diffusion Tensor Imaging Tractography. Cereb Cortex 19:524–536. doi: 10.1093/cercor/bhn102

Gundelach J, Koch M (2018) Redirection of neuroblast migration from the rostral migratory stream into a lesion in the prefrontal cortex of adult rats. Exp Brain Res 236:1181–1191. doi: 10.1007/s00221-018-5209-3

Gupta R, Trivedi R, Rathore R (2008) Review: Clinical application of diffusion tensor imaging. Indian J Radiol Imaging 18:45. doi: 10.4103/0971-3026.38505

Hack I, Bancila M, Loulier K, et al (2002) Reelin is a detachment signal in tangential chain-migration during postnatal neurogenesis. Nat Neurosci 5:939–945. doi: 10.1038/nn923

Haglid KG, Wang S, Qiner Y, Hamberger A (1994) Excitotoxicity. Mol Neurobiol 9:259–263. doi: 10.1007/BF02816125

Hara T, Nakamura K, Matsui M, et al (2006) Suppression of basal autophagy in neural cells causes neurodegenerative disease in mice. Nature 441:885–889. doi: 10.1038/nature04724

Hatten ME (1999) Central Nervous System Neuronal Migration. Annu Rev Neurosci 22:511–539. doi: 10.1146/annurev.neuro.22.1.511

Heidbreder CA, Groenewegen HJ (2003) The medial prefrontal cortex in the rat: Evidence for a dorso-ventral distinction based upon functional and anatomical characteristics. Neurosci Biobehav Rev 27:555–579. doi: 10.1016/j.neubiorev.2003.09.003

Hildebrandt H, Mühlenhoff M, Weinhold B, Gerardy-Schahn R (2007) Dissecting polysialic acid and NCAM functions in brain development. J Neurochem 103 Suppl:56–64. doi: 10.1111/j.1471-4159.2007.04716.x

Hoehn M, Küstermann E, Blunk J, et al (2002) Monitoring of implanted stem cell migration in vivo: a highly resolved in vivo magnetic resonance imaging investigation of experimental stroke in rat. Proc Natl Acad Sci U S A 99:16267–72. doi: 10.1073/pnas.242435499

Hu H, Tomasiewicz H, Magnuson T, Rutishauser U (1996) The role of polysialic acid in migration of olfactory bulb interneuron precursors in the subventricular zone. Neuron 16:735–743. doi: 10.1016/S0896-6273(00)80094-X

Jablonska B, Aguirre A, Raymond M, et al (2010) Chordin-induced lineage plasticity of adult SVZ neuroblasts after demyelination. Nat Neurosci 13:541–550. doi: 10.1038/nn.2536

Jankovic J (2008) Parkinson's disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry 79:368–76. doi: 10.1136/jnnp.2007.131045

Jankovski A, Garcia C, Soriano E, Sotelo C (1998) Proliferation, migration and differentiation of neuronal progenitor cells in the adult mouse subventricular zone surgically separated from its olfactory bulb. Eur J Neurosci 10:3853–3868. doi: 10.1046/j.1460-9568.1998.00397.x

Jarrard LE (1989) On the use of ibotenic acid to lesion selectively different components of the hippocampal formation. J Neurosci Methods 29:251–259. doi: 10.1016/0165-0270(89)90149-0

Jin K, Sun Y, Xie L, et al (2003) Directed migration of neuronal precursors into the ischemic cerebral cortex and striatum. Mol Cell Neurosci 24:171–189. doi: 10.1016/S1044-7431(03)00159-3

Kang BK, Na DG, Ryoo JW, et al (2001) Diffusion-weighted MR imaging of intracerebral hemorrhage. Korean J Radiol 2:183–91. doi: 10.3348/kjr.2001.2.4.183

Kim WR, Kim Y, Eun B, et al (2007) Impaired Migration in the Rostral Migratory Stream But Spared Olfactory Function after the Elimination of Programmed Cell Death in Bax Knock-Out Mice. J Neurosci 27:14392–14403. doi: 10.1523/JNEUROSCI.3903-07.2007

Kirschenbaum B, Doetsch F, Lois C, Alvarez-Buylla A (1999) Adult subventricular zone neuronal precursors continue to proliferate and migrate in the absence of the olfactory bulb. J Neurosci 19:2171–2180. doi: 10.1523/jneurosci.2751-04.2004

Kleim JA, Jones TA (2008) Principles of Experience-Dependent Neural Plasticity: Implications for Rehabilitation After Brain Damage. J Speech Lang Hear Res 51:S225. doi: 10.1044/1092-4388(2008/018)

Klionsky DJ, Emr SD (2000) Autophagy as a regulated pathway of cellular degradation. Science (80-) 290:1717–1721. doi: 10.1126/science.290.5497.1717

Koizumi H, Higginbotham H, Poon T, et al The microtubule protein Doublecortin contributes to maintenance of bipolar shape and nuclear translocation during neuronal migration in the adult forebrain

Kolb B, Nonneman AJ, Singh RK (1974) Double dissociation of spatial impairments and perseveration following selective prefrontal lesions in rats. J Comp Physiol Psychol 87:772–80

Kornack DR, Rakic P (2001) The generation, migration, and differentiation of olfactory neurons in the adult primate brain. Proc Natl Acad Sci USA 98:4752–4757. doi: 10.1073/pnas.081074998

Kuhn H, Dickinson-Anson H, Gage F (1996) Neurogenesis in the dentate gyrus of the adult rat: age-related decrease of neuronal progenitor proliferation. J Neurosci 16:2027–2033. doi: 10.1523/JNEUROSCI.16-06-02027.1996

Kulbe JR, Hall ED (2017) Chronic traumatic encephalopathy-integration of canonical traumatic brain injury secondary injury mechanisms with tau pathology. Prog Neurobiol 158:15–44. doi: https://doi.org/10.1016/j.pneurobio.2017.08.003

Kuma A, Hatano M, Matsui M, et al (2004) The role of autophagy during the early neonatal starvation period. Nature 432:1032–1036. doi: 10.1038/nature03029

Kunze A, Achilles A, Keiner S, et al (2015) Two distinct populations of doublecortin-positive cells in the perilesional zone of cortical infarcts. BMC Neurosci 16:20. doi: 10.1186/s12868-015-0160-8

Lacroix L, Spinelli S, Heidbreder CA, Feldon J (2000) Differential role of the medial and lateral prefrontal cortices in fear and anxiety. Behav Neurosci 114:1119–1130. doi: 10.1037/0735-7044.114.6.1119

Landmesser L, Dahm L, Tang J, Rutishauser U (1990) Polysialic acid as a regulator of intramuscular nerve branching during embryonic development. Neuron 4:655–667. doi: 10.1016/0896-6273(90)90193-J

Laviola G, Hannan AJ, Macrì S, et al (2008) Effects of enriched environment on animal models of neurodegenerative diseases and psychiatric disorders. Neurobiol Dis 31:159–168. doi: 10.1016/J.NBD.2008.05.001

Le Bihan D, Mangin JF, Poupon C, et al (2001) Diffusion tensor imaging: concepts and applications. J Magn Reson Imaging 13:534–546. doi: 10.1002/jmri.1076

Le Bihan D, Poupon C, Amadon A, Lethimonnier F (2006) Artifacts and pitfalls in diffusion MRI. J Magn Reson Imaging 24:478–488. doi: 10.1002/jmri.20683

Leavitt BR, Hernit-Grant CS, Macklis JD (1999) Mature astrocytes transform into transitional radial glia within adult mouse neocortex that supports directed migration of transplanted immature neurons. Exp Neurol 157:43–57. doi: 10.1006/exnr.1999.6982

Lee S-R (2006) Involvement of Matrix Metalloproteinase in Neuroblast Cell Migration from the Subventricular Zone after Stroke. J Neurosci 26:3491–3495. doi: 10.1523/JNEUROSCI.4085-05.2006

Leong SY, Turnley AM (2011) Regulation of adult neural precursor cell migration. Neurochem Int 59:382–393. doi: 10.1016/j.neuint.2010.12.024

Li B, Piao C-S, Liu X-Y, et al (2010) Brain self-protection: The role of endogenous neural progenitor cells in adult brain after cerebral cortical ischemia. Brain Res 1327:91–102. doi: 10.1016/j.brainres.2010.02.030

Li L, Jiang W, Luo K, et al (2013) Superparamagnetic iron oxide nanoparticles as MRI contrast agents for non-invasive stem cell labeling and tracking. Theranostics 3:595–615. doi: 10.7150/thno.5366

Liu L, Rudin M, Kozlova EN (2000) Glial cell proliferation in the spinal cord after dorsal rhizotomy or sciatic nerve transection in the adult rat. Exp brain Res 131:64–73

Lo EH, Moskowitz MA, Jacobs TP (2005) Exciting, Radical, Suicidal. Stroke 36:189–192. doi: 10.1161/01.STR.0000153069.96296.fd

Lois C, Alvarez-Buylla A (1994) Long-distance neuronal migration in the adult mammalian brain. Science (80-) 264:1145–1148. doi: 10.1126/science.8178174

Lois C, Garcia-Verdugo JM, Alvarez-Buylla A (1996) Chain migration of neuronal precursors. Science (80-) 271:978–981

Los M, Mozoluk M, Ferrari D, et al (2002) Activation and Caspase-mediated Inhibition of PARP: A Molecular Switch between Fibroblast Necrosis and Apoptosis in Death Receptor Signaling. Mol Biol Cell 13:978–988. doi: 10.1091/mbc.01-05-0272

Luo L (2000) Rho GTPases in neuronal morphogenesis. Nat Rev Neurosci 1:173–180. doi: 10.1038/35044547

Maas AIR, Stocchetti N, Bullock R (2008) Moderate and severe traumatic brain injury in adults. Lancet Neurol 7:728–741. doi: https://doi.org/10.1016/S1474-4422(08)70164-9

Marín O (2013) Cellular and molecular mechanisms controlling the migration of neocortical interneurons. Eur J Neurosci 38:2019–2029. doi: 10.1111/ejn.12225

Marín O, Rubenstein JLR (2003) Cell migration in the forebrain. Annu Rev Neurosci 26:441–483. doi: 10.1146/annurev.neuro.26.041002.131058

Martinez-Molina N, Kim Y, Hockberger P, Szele FG (2011) Rostral migratory stream neuroblasts turn and change directions in stereotypic patterns. Cell Adhes Migr 5:83–95. doi: 10.4161/cam.5.1.13788

Mattson MP, Chan SL (2003) Calcium orchestrates apoptosis. Nat Cell Biol 5:1041–1043. doi: 10.1038/ncb1203-1041

Memberg SP, Hall AK (1995) Proliferation, differentiation, and survival of rat sensory neuron precursors in vitro require specific trophic factors. Mol Cell Neurosci 6:323–335. doi: 10.1006/mcne.1995.1025

Meyer AK, Maisel M, Hermann A, et al (2010) Restorative approaches in Parkinson's Disease: Which cell type wins the race? J Neurol Sci 289:93–103. doi: 10.1016/j.jns.2009.08.024

Ming G li, Song H (2011) Adult Neurogenesis in the Mammalian Brain: Significant Answers and Significant Questions. Neuron 70:687–702. doi: 10.1016/j.neuron.2011.05.001

Mitchell JB, Laiacona J (1998) The medial frontal cortex and temporal memory: tests using spontaneous exploratory behaviour in the rat. Behav Brain Res 97:107–113. doi: http://dx.doi.org/10.1016/S0166-4328(98)00032-1

Mizrahi A, Lu J, Irving R, et al (2006) In vivo imaging of juxtaglomerular neuron turnover in the mouse olfactory bulb. Proc Natl Acad Sci 103:1912–1917. doi: 10.1073/pnas.0506297103

Mizushima N, Levine B, Cuervo AM, Klionsky DJ (2008) Autophagy fights disease through cellular self-digestion. Nature 451:1069–1075. doi: 10.1038/nature06639

Mouret A, Lepousez G, Gras J, et al (2009) Turnover of Newborn Olfactory Bulb Neurons Optimizes Olfaction. J Neurosci 29:12302–12314. doi: 10.1523/JNEUROSCI.3383-09.2009

Mozdziak P, Schultz E (2000) Retroviral labeling is an appropriate marker for dividing cells. Biotech Histochem 75:141–146. doi: 10.3109/10520290009066492

Muir JL, Everitt BJ, Robbins TW (1996) The cerebral cortex of the rat and visual attentional function: Dissociable effects of mediofrontal, cingulate, anterior dorsolateral, and parietal cortex lesions on a five-choice serial reaction time task. Cereb Cortex 6:470–481. doi: 10.1093/cercor/6.3.470

Muller D, Wang C, Skibo G, et al (1996) PSA-NCAM is required for activity-induced synaptic plasticity. Neuron 17:413–422. doi: 10.1016/S0896-6273(00)80174-9

Nam SC, Kim Y, Dryanovski D, et al (2007) Dynamic features of postnatal subventricular zone cell motility: A two-photon time-lapse study. J Comp Neurol 505:190–208. doi: 10.1002/cne.21473

Ng KL, Li J-D, Cheng MY, et al (2005) Dependence of olfactory bulb neurogenesis on prokineticin 2 signaling. Science 308:1923–7. doi: 10.1126/science.1112103

Nguyen-Ba-Charvet KT (2004) Multiple Roles for Slits in the Control of Cell Migration in the Rostral Migratory Stream. J Neurosci 24:1497–1506. doi: 10.1523/JNEUROSCI.4729-03.2004

Nikoletopoulou V, Markaki M, Palikaras K, Tavernarakis N (2013) Crosstalk between apoptosis, necrosis and autophagy. Biochim Biophys Acta - Mol Cell Res 1833:3448–3459. doi: 10.1016/J.BBAMCR.2013.06.001

Nithianantharajah J, Hannan AJ (2011) Mechanisms mediating brain and cognitive reserve: Experience-dependent neuroprotection and functional compensation in animal models of neurodegenerative diseases. Prog Neuro-Psychopharmacology Biol Psychiatry 35:331–339. doi: 10.1016/j.pnpbp.2010.10.026

Novak MJU, Tabrizi SJ (2010) Huntington's disease. BMJ 340:c3109. doi: 10.1136/BMJ.C3109

Nucifora PGP, Verma R, Lee S-K, Melhem ER (2007) Diffusion-Tensor MR Imaging and Tractography: Exploring Brain Microstructure and Connectivity. Radiology 245:367–384. doi: 10.1148/radiol.2452060445

Nudo RJ (2013) Recovery after brain injury: mechanisms and principles. Front Hum Neurosci 7:887. doi: 10.3389/fnhum.2013.00887

O'Rourke NA, Sullivan DP, Kaznowski CE, et al (1995) Tangential migration of neurons in the developing cerebral cortex. Development 121:2165–2176. doi: 10.1093/intimm/dxr096

Olanow CW, Agid Y, Mizuno Y, et al (2004) Levodopa in the treatment of Parkinson's disease: Current controversies. Mov Disord 19:997–1005. doi: 10.1002/mds.20243

Omori Y, Suzuki M, Ozaki K, et al (1998) Expression and chromosomal localization of KIAA0369, a putative kinase structurally related to Doublecortin. J Hum Genet 43:169–177. doi: 10.1007/s100380050063

Ono K, Tomasiewicz H, Magnuson T, Rutishauser U (1994) N-CAM mutation inhibits tangential neuronal migration and is phenocopied by enzymatic removal of polysialic acid. Neuron 13:595–609. doi: 10.1016/0896-6273(94)90028-0

Paratcha G, Ibanez CF, Ledda F (2006) GDNF is a chemoattractant factor for neuronal precursor cells in the rostral migratory stream. Mol Cell Neurosci 31:505–514. doi: 10.1016/j.mcn.2005.11.007

Parent JM, Yu TW, Leibowitz RT, et al (1997) Dentate Granule Cell Neurogenesis Is Increased by Seizures and Contributes to Aberrant Network Reorganization in the Adult Rat Hippocampus. J Neurosci 17:3727–3738

Park HT, Wu J, Rao Y (2002) Molecular control of neuronal migration. BioEssays 24:821–827. doi: 10.1002/bies.10141

Pekcec A, Mühlenhoff M, Gerardy-Schahn R, Potschka H (2007) Impact of the PSA-NCAM system on pathophysiology in a chronic rodent model of temporal lobe epilepsy. Neurobiol Dis 27:54–66. doi: 10.1016/j.nbd.2007.04.002

Peretto P, Merighi A, Fasolo A, Bonfanti L (1997) Glial Tubes in the Rostral Migratory Stream of the Adult Rat. Brain Res Bull 42:9–21. doi: 10.1016/S0361-9230(96)00116-5

Petreanu L, Alvarez-Buylla A (2002) Maturation and Death of Adult-Born Olfactory Bulb Granule Neurons: Role of Olfaction. J Neurosci 22:6106–6113. doi: 10.1523/JNEUROSCI.22-14-06106.2002

Petridis AK, El Maarouf A, Rutishauser U (2004) Polysialic acid regulates cell contact-dependent neuronal differentiation of progenitor cells from the subventricular zone. Dev Dyn 230:675–684. doi: 10.1002/dvdy.20094

Pettus EH, Christman CW, Giebel ML, Povlishock JT (1994) Traumatically Induced Altered Membrane Permeability: Its Relationship to Traumatically Induced Reactive Axonal Change. J Neurotrauma 11:507–522. doi: 10.1089/neu.1994.11.507

Pini L, Pievani M, Bocchetta M, et al (2016) Brain atrophy in Alzheimer's Disease and aging. Ageing Res Rev 30:25–48. doi: 10.1016/J.ARR.2016.01.002

Pothayee N, Cummings DM, Schoenfeld TJ, et al (2017) Magnetic resonance imaging of odorant activity-dependent migration of neural precursor cells and olfactory bulb growth. Neuroimage 158:232–241. doi: 10.1016/j.neuroimage.2017.06.060

Povlishock JT, Katz DI (2005) Update of neuropathology and neurological recovery after traumatic brain injury. J Head Trauma Rehabil 20:76–94

Prosser HM, Bradley A, Caldwell MA (2007) Olfactory bulb hypoplasia in Prokr2 null mice stems from defective neuronal progenitor migration and differentiation. Eur J Neurosci 26:3339–44. doi: 10.1111/j.1460-9568.2007.05958.x

Pudenz RH, Shelden CH (1946) The Lucite Calvarium—A Method for Direct Observation of the Brain. J Neurosurg 3:487–505. doi: 10.3171/jns.1946.3.6.0487

Quirk GJ, Beer JS (2006) Prefrontal involvement in the regulation of emotion: convergence of rat and human studies. Curr Opin Neurobiol 16:723–727. doi: 10.1016/J.CONB.2006.07.004

Ramos-Moreno T, Galazo MJ, Porrero C, et al (2006) Extracellular matrix molecules and synaptic plasticity: immunomapping of intracellular and secreted Reelin in the adult rat brain. Eur J Neurosci 23:401–422. doi: 10.1111/j.1460-9568.2005.04567.x

Reynolds BA, Weiss S (1992) Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science (80-) 255:1707–1710. doi: 10.1126/science.1553558

Robin AM, Zhang ZG, Wang L, et al (2006) Stromal Cell-Derived Factor 1α Mediates Neural Progenitor Cell Motility after Focal Cerebral Ischemia. J Cereb Blood Flow Metab 26:125–134. doi: 10.1038/sj.jcbfm.9600172

Roos RA, Hermans J, Vegter-van der Vlis M, et al (1993) Duration of illness in Huntington's disease is not related to age at onset. J Neurol Neurosurg Psychiatry 56:98–100. doi: 10.1136/JNNP.56.1.98

Rousselot P, Lois C, Alvarez-Buylla A (1995) Embryonic (PSA) N-CAM reveals chains of migrationg neuroblasts between the lateral ventricle and the olfactory bulb of adult mice. J Comp Neurol 351:51–61. doi: 10.1002/cne.903510106

Sanai N, Nguyen T, Ihrie RA, et al (2011) Corridors of migrating neurons in the human brain and their decline during infancy. Nature 478:382–386. doi: 10.1038/nature10487

Sanai N, Tramontin AD, Quinones-Hinojosa A, et al (2004) Unique astrocyte ribbon in adult human brain contains neural stem cells but lacks chain migration. Nature 427:740–744. doi: 10.1038/nature02301

Sánchez-Santed F, De Bruin JPC, Heinsbroek RPW, Verwer RWH (1997) Spatial delayed alternation of rats in a T-maze: Effects of neurotoxic lesions of the medial prefrontal cortex and of T-maze rotations. Behav Brain Res 84:73–79. doi: 10.1016/S0166-4328(97)83327-X

Sbardella E, Tona F, Petsas N, Pantano P (2013) DTI Measurements in Multiple Sclerosis: Evaluation of Brain Damage and Clinical Implications. Mult Scler Int 2013:. doi: 10.1155/2013/671730

Schindelin J, Arganda-Carreras I, Frise E, et al (2012) Fiji: An open-source platform for biological-image analysis. Nat Methods 9:676–682. doi: 10.1038/nmeth.2019

Schindelin J, Rueden CT, Hiner MC, Eliceiri KW (2015) The ImageJ ecosystem: An open platform for biomedical image analysis. Mol Reprod Dev 82:518–529. doi: 10.1002/mrd.22489

Schneider CA, Rasband WS, Eliceiri KW (2012) NIH Image to ImageJ: 25 years of image analysis. Nat Methods 9:671–675. doi: 10.1038/nmeth.2089

Seamans JK, Floresco SB, Phillips AG (1995) Functional differences between the prelimbic and anterior cingulate regions of the rat prefrontal cortex. Behav Neurosci 109:1063–73

Seidenfaden R, Krauter A, Schertzinger F, et al (2003) Polysialic Acid Directs Tumor Cell Growth by Controlling Heterophilic Neural Cell Adhesion Molecule Interactions. Mol Cell Biol 23:5908–5918. doi: 10.1128/MCB.23.16.5908-5918.2003

Seki T, Rutishauser U (1998) Removal of polysialic acid-neural cell adhesion molecule induces aberrant mossy fiber innervation and ectopic synaptogenesis in the hippocampus. J Neurosci 18:3757–3766. doi: 10.1523/JNEUROSCI.18-10-03757.1998

Senkov O, Sun M, Weinhold B, et al (2006) Polysialylated Neural Cell Adhesion Molecule Is Involved in Induction of Long-Term Potentiation and Memory Acquisition and Consolidation in a Fear-Conditioning Paradigm. J Neurosci 26:10888–109898. doi: 10.1523/JNEUROSCI.0878-06.2006

Shapiro EM, Medford-Davis LN, Fahmy TM, et al (2007) Antibody-mediated cell labeling of peripheral T cells with micron-sized iron oxide particles (MPIOs) allows single cell detection by MRI. Contrast Media Mol Imaging 2:147–153. doi: 10.1002/cmmi.134

Shin JJ, Fricker-Gates RA, Perez FA, et al (2000) Transplanted Neuroblasts Differentiate Appropriately into Projection Neurons with Correct Neurotransmitter and Receptor Phenotype in Neocortex Undergoing Targeted Projection Neuron Degeneration. J Neurosci 20:7404–7416

Shook BA, Manz DH, Peters JJ, et al (2012) Spatiotemporal Changes to the Subventricular Zone Stem Cell Pool through Aging. J Neurosci 32:6947–6956. doi: 10.1523/JNEUROSCI.5987-11.2012

Sit S-T, Manser E (2011) Rho GTPases and their role in organizing the actin cytoskeleton. J Cell Sci 124:679–83. doi: 10.1242/jcs.064964

Skandsen T, Kvistad KA, Solheim O, et al (2010) Prevalence and impact of diffuse axonal injury in patients with moderate and severe head injury: a cohort study of early magnetic resonance imaging findings and 1-year outcome. J Neurosurg 113:556–563. doi: 10.3171/2009.9.jns09626

Snyder JS (2018) Questioning human neurogenesis. Nature 555:315–316. doi: 10.1038/d41586-018-02629-3

Soen Y, Mori A, Palmer TD, Brown PO (2006) Exploring the regulation of human neural precursor cell differentiation using arrays of signaling microenvironments. Mol Syst Biol 2:37. doi: 10.1038/msb4100076

Sorrells SF, Paredes MF, Cebrian-Silla A, et al (2018) Human hippocampal neurogenesis drops sharply in children to undetectable levels in adults. Nature 555:377–381. doi: 10.1038/nature25975

Spreux-Varoquaux O, Bensimon G, Lacomblez L, et al (2002) Glutamate levels in cerebrospinal fluid in amyotrophic lateral sclerosis: a reappraisal using a new HPLC method with coulometric detection in a large cohort of patients. J Neurol Sci 193:73–8

Steindler DA, Laywell ED (2003) Astrocytes as stem cells: Nomenclature, phenotype, and translation. Glia 43:62–69. doi: 10.1002/glia.10242

Stocchetti N, Taccone FS, Citerio G, et al (2015) Neuroprotection in acute brain injury: an up-to-date review. Crit Care 19:186. doi: 10.1186/s13054-015-0887-8

Stys PK, Waxman SG, Ransom BR (1992) Ionic mechanisms of anoxic injury in mammalian CNS white matter: role of Na+ channels and Na(+)-Ca2+ exchanger. J Neurosci 12:430–9. doi: 10.1523/JNEUROSCI.12-02-00430.1992

Su Z, Niu W, Liu M-L, et al (2014) In vivo conversion of astrocytes to neurons in the injured adult spinal cord. Nat Commun 5:3338. doi: 10.1038/ncomms4338

Sullivan RM, Gratton A (2002) Behavioral effects of excitotoxic lesions of ventral medial prefrontal cortex in the rat are hemisphere-dependent. Brain Res 927:69–79. doi: 10.1016/S0006-8993(01)03328-5

Sun W, Kim H, Moon Y (2010) Control of neuronal migration through rostral migration stream in mice. Anat Cell Biol 43:269. doi: 10.5115/acb.2010.43.4.269

Szydlowska K, Tymianski M (2010) Calcium, ischemia and excitotoxicity. Cell Calcium 47:122–129

Tang J, Landmesser L, Rutishauser U (1992) Polysialic acid influences specific pathfinding by avian motoneurons. Neuron 8:1031–1044. doi: 10.1016/0896-6273(92)90125-W

Tang J, Rutishauser U, Landmesser L (1994) Polysialic acid regulates growth cone behavior during sorting of motor axons in the plexus region. Neuron 13:405–414. doi: 10.1016/0896-6273(94)90356-5

Taupin P (2007) BrdU immunohistochemistry for studying adult neurogenesis: Paradigms, pitfalls, limitations, and validation. Brain Res Rev 53:198–214. doi: 10.1016/j.brainresrev.2006.08.002

Taylor CA, Bell JM, Breiding MJ, Xu L (2017) Traumatic Brain Injury–Related Emergency Department Visits, Hospitalizations, and Deaths — United States, 2007 and 2013. MMWR Surveill Summ 66:1–16. doi: 10.15585/mmwr.ss6609a1

Tropepe V, Craig CG, Morshead CM, van der Kooy D (1997) Transforming Growth Factor-α Null and Senescent Mice Show Decreased Neural Progenitor Cell Proliferation in the Forebrain Subependyma. J Neurosci 17:7850–7859. doi: 10.1523/JNEUROSCI.17-20-07850.1997

Tsai L-H, Gleeson JG (2005) Nucleokinesis in neuronal migration. Neuron 46:383–8. doi: 10.1016/j.neuron.2005.04.013

Uylings HBM, Groenewegen HJ, Kolb B (2003) Do rats have a prefrontal cortex? Behav Brain Res 146:3–17. doi: 10.1016/j.bbr.2003.09.028

Valdés-Hernández PA, Sumiyoshi A, Nonaka H, et al (2011) An in vivo MRI Template Set for Morphometry, Tissue Segmentation, and fMRI Localization in Rats. Front Neuroinform 5:26. doi: 10.3389/fninf.2011.00026

Vargas ME, Barres BA (2007) Why Is Wallerian Degeneration in the CNS So Slow? Annu Rev Neurosci 30:153–179. doi: 10.1146/annurev.neuro.30.051606.094354

Verwer RWH, Sluiter AA, Balesar RA, et al (2007) Mature astrocytes in the adult human neocortex express the early neuronal marker doublecortin. Brain 130:3321–3335. doi: 10.1093/brain/awm264

Vos T, Allen C, Arora M, et al (2016) Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. Lancet 388:1545–1602. doi: 10.1016/S0140-6736(16)31678-6

Waller A (1850) Experiments on the Section of the Glossopharyngeal and Hypoglossal Nerves of the Frog, and Observations of the Alterations Produced Thereby in the Structure of Their Primitive Fibres. Philos Trans R Soc London 140:423–429. doi: 10.1098/rstl.1850.0021

Wang C, Liu F, Liu Y-Y, et al (2011) Identification and characterization of neuroblasts in the subventricular zone and rostral migratory stream of the adult human brain. Cell Res 21:1534–1550. doi: 10.1038/cr.2011.83

Wang C, Youle RJ (2009) The role of mitochondria in apoptosis. Annu Rev Genet 43:95–118. doi: 10.1146/annurev-genet-102108-134850

Wang Y, Shi Y, Wei H (2017) Calcium Dysregulation in Alzheimer's Disease: A Target for New Drug Development. J Alzheimer's Dis Park 7:. doi: 10.4172/2161-0460.1000374

Wanner IB, Anderson MA, Song B, et al (2013) Glial Scar Borders Are Formed by Newly Proliferated, Elongated Astrocytes That Interact to Corral Inflammatory and Fibrotic Cells via STAT3-Dependent Mechanisms after Spinal Cord Injury. J Neurosci 33:12870–12886. doi: 10.1523/jneurosci.2121-13.2013

Ward M, McCann C, DeWulf M, et al (2003) Distinguishing between directional guidance and motility regulation in neuronal migration. J Neurosci 23:5170–7

Wheeler R Extended Depth of Field. http://www.richardwheeler.net/contentpages/text.php?gallery=ImageJ_Macros&file=Extended_Depth_of_Field&type=ijm. Accessed 6 Sep 2018

Whitman MC, Fan W, Rela L, et al (2009) Blood vessels form a migratory scaffold in the rostral migratory stream. J Comp Neurol 516:94–104. doi: 10.1002/cne.22093

Wichterle H, García-Verdugo JM, Alvarez-Buylla A (1997) Direct evidence for homotypic, glia-independent neuronal migration. Neuron 18:779–791. doi: 10.1016/S0896-6273(00)80317-7

Wiegell MR, Larsson HBW, Wedeen VJ (2000) Fiber Crossing in Human Brain Depicted with Diffusion Tensor MR Imaging1. Radiology 217:897–903

Wikmark RGE, Divac I, Weiss R (1973) Retention of Spatial Delayed Alternation in Rats with Lesions in the Frontal Lobes. Brain Behav Evol 8:329–339. doi: 10.1159/000124362

Wojtowicz JM, Kee N (2006) BrdU assay for neurogenesis in rodents. Nat Protoc 1:1399–1405. doi: 10.1038/nprot.2006.224

Wu W, Wong K, Chen J, et al (1999) Directional guidance of neuronal migration in the olfactory system by the protein Slit. Nature 400:331–6. doi: 10.1038/22477

Xu Q, Wang S, Jiang X, et al (2007) Hypoxia-induced astrocytes promote the migration of neural progenitor cells via vascular endothelial factor, stem cell factor, stromal-derived factor-1α and monocyte chemoattractant protein-1 upregulation in vitro. Clin Exp Pharmacol Physiol 34:624–631. doi: 10.1111/j.1440-1681.2007.04619.x

Yan Y-P, Sailor KA, Lang BT, et al (2007) Monocyte Chemoattractant Protein-1 Plays a Critical Role in Neuroblast Migration after Focal Cerebral Ischemia. J Cereb Blood Flow Metab 27:1213–1224. doi: 10.1038/sj.jcbfm.9600432

Yi J-H, Hazell AS (2006) Excitotoxic mechanisms and the role of astrocytic glutamate transporters in traumatic brain injury. Neurochem Int 48:394–403. doi: 10.1016/J.NEUINT.2005.12.001

Zinkand WC, Moore WC, Thompson C, et al (1992) Ibotenic acid mediates neurotoxicity and phosphoinositide hydrolysis by independent receptor mechanisms. Mol Chem Neuropathol 16:1–10. doi: 10.1007/BF03159956

Zündorf G, Reiser G (2011) Calcium dysregulation and homeostasis of neural calcium in the molecular mechanisms of neurodegenerative diseases provide multiple targets for neuroprotection. Antioxid Redox Signal 14:1275–88. doi: 10.1089/ars.2010.3359

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