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DISSERTATION

Intrahippocampal transplantation of MSC and MSC-expressing BDNF in Rat Models of Depression-like Behaviour

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List of Abbreviations

1W-ANOVA: one Way-Analysis of Variance
5-HIAA: 5-hydroxyindolacetic acid
5-HT: 5-Hydroxy-Tryptamine (Serotonin)
A: active avoidance
ACTH: Adrenocorticotropine Hormone
AD: Antidepressant Drugs
BDNF: Brain-Derived Neurotrophic Factor
BM: Bone Marrow
BrdU: 5-Bromo-2'-deoxyuridin
CNS: Central Nervous System
CREB: cAMP response element-binding protein
CRF: Corticotropine Releasing Factor
DAPI: 4',6' Di Amidino-2-Phenyl Indole
DG: Dentate Gyrus
DSM-IV: Diagnostic and Statistical Manual of Mental Disorders-Fourth Edition
ECT: Electroconvulsive Therapy
EM: Elevated Plus Maze
FACS: Fluorescence-Activated Cell Sorting
FST: Forced Swim Test
GCL: Granular Cell Layer
GFP: Green Fluorescent Protein
HPA: Hypothalamus-Pituitary-Adrenal
Iba1: Ionized calcium-binding adaptor molecule 1
i.c: intracranial
i.p.: intraperitoneal
ICD 10: International Classification of Diseases-Tenth Edition
IdU: 5-Iodo-2'-deoxyuridin
IRES: Internal Ribosome Entry Site
IS: Inescapable shock session
LH: Learned Helplessness
LTP: Long Term Potentiation

LV-GFP-BDNF: Lentivirus encoding enhanced GFP and BDNF with IRES

LV-GFP: Lentivirus encoding enhanced GFP

mA: milli Ampere

MAOI: Mono Amine Oxidase-Inhibitor

MD: Major Depression

m.o.i.: Multiplicity Of Infection

MSC: Mesenchymal Stromal Cell

MSC-BDNF: MSC transduced with LV-GFP-BDNF

MSC-GFP: MSC transduced with LV-GFP

MSC-VSOP: MSC labelled with VSOP

MRI: Magnetic Resonance Imaging

NE: Norepinephrine

NGF: Nerve Growth Factor

NMR: Nuclear Magnetic Resonance

NSF: Novelty-Suppressed Feeding

NT: Neurotrophin

OF: Open Field

OSST: Open Space Swim Test

PBS: Phosphate-Buffered Saline

PET: Positron Emission Tomography

PFA: Paraformaldehyde

PFC: Prefrontal Cortex

PVN: Paraventricular Nucleus

RAM: 8-arm Radial Maze

RM-ANOVA: Repeated Measurement-Analysis of Variance

RME: Reference Memory Error

SGZ: Sub-Granular Zone

SSRI: Selective Serotonin Reuptake Inhibitor

SPIO: Superparamagnetic Iron Oxide Particles

SPT: Sucrose Preference Test

SVZ: Sub-Ventricular Zone

T: Tesla

TE: Echo Time

TR: Repetition Time

TST: Tail Suspension Test

TrkB: Tyrosine kinase B receptor

USPIO: Ultra Small superParamagnetic Iron 0xide

VSOP: Very small superparamagnetic particles

WME: Working Memory Error

I. Introduction

1. Depression(s)

1.1. General

Mood disorders are among the most prevalent, recurrent, and disabling neuropsychiatric disorders. They include several forms of Depression, such as Major Depression, Bipolar Disorder and Dysthymic Disorder. Among them, Major Depression (MD) is the most serious disease with a genetic predisposition factor of about 40 %. MD affects approximately 17% of the population at some point in life. Among depressed patients, 15% are dying by suicide (Souery et al. 2006). Depression has been projected to become the second leading cause of disability worldwide by 2020, resulting in major social and economic consequences (Murray et al. 2006). Table 1 summarizes the main symptoms of MD according to the two diagnosistic references: the Diagnostic and Statistical Manual of Mental Disorders- Fourth Edition (DSM-IV) of the American Psychiatric Association (1994) and the International Classification of Diseases-Tenth Edition (ICD-10). Those classifications are more guidelines than categorizing methods because of overlapping symptoms with others mood disorder, such as anxiety.

There are several therapeutic approaches for the treatment of MD. The main one is based on the monoamine hypothesis of Depression and involves an increase of intrasynaptic Serotonin levels by medication of Antidepressant Drugs (ADs). However, the most effective treatment is the Electroconvulsive Therapy (ECT). With a remission rate of nearly 80%, ECT is nevertheless applied as a second-line treatment due to its traumatising character. The mechanisms of action of ECT are still being investigated. All therapeutic approaches can be associated with psychotherapy, which in some cases is efficient on its own.

Table 1: Main symptoms present in Major Depression

Recurrent thoughts of death, recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide
Anhedonia: loss of interest or pleasure in things that used to be fun
Diminished ability to think or concentrate, or indecisiveness, nearly every day
Irritability, hostility, aggression
A decrease in the ability to make decisions
Feeling hopeless or helpless
Feeling restless or unable to sit still
Loss of energy
Withdrawal from family members/peers/co-workers
Changes in appetite or weight
Drug or alcohol abuse
Trouble sleeping or sleeping too much
Blaming yourself for things going wrong
Crying a lot

One major issue in MD is the absence of a curative treatment. In 29 to 46 % of cases, full remission cannot be achieved (Fava and Davidson 1996). Therefore, our understanding of the biology of MD needs to be improved in order to develop new therapeutic strategies (Wong and Licinio 2004). Researches are currently exploring new approaches based on the neurogenesis and the neurotrophin hypothesis of Depression.

1.2. Etiology of Depression: stress and inflammation

Stressful life events, such as intolerable daily work conditions, mate separation, financial problems, have a substantial association with all kinds of Depression. Risk factors for some stressful life events are correlated to genetic predispositions to major Depression (Kendler and Karkowski-Shuman 1997). One major key pathway in stress is the Hypothalamus-Pituitary-Adrenal (HPA) axis, which is highly activated in MD patients (Nemeroff 1996; Holsboer 2000). Figure 1 represents the HPA axis regulation in the human under stress (Nestler et al. 2002). Stressful events integrated from the hippocampus and amygdala result in the release of the Corticotropine-Releasing Factor (CRF) from the Paraventricular Nucleus (PVN) of the hypothalamus. CRF induces the synthesis of the Adrenocorticotropine Hormone (ACTH) in the anterior pituitary, which is

released into the blood stream. ACTH then induces the synthesis and release of glucocorticoids, among which corticosterol, the so-called “stress hormone”. Glucocorticoids act in a negative feedback way on the hippocampal formation, which exhibits the highest density of Glucortorticoid Receptors (GR). Under normal conditions, this feedback loop inhibits the release of CRF from the PVN and stops the stress experience. With repeated stress events, this loop does not act properly and results in the neurobiological disturbances observed in MD patients, such as impaired hippocampal function accompanied by reduced hippocampal volume (MacQueen et al. 2003; Squire et al. 2004).

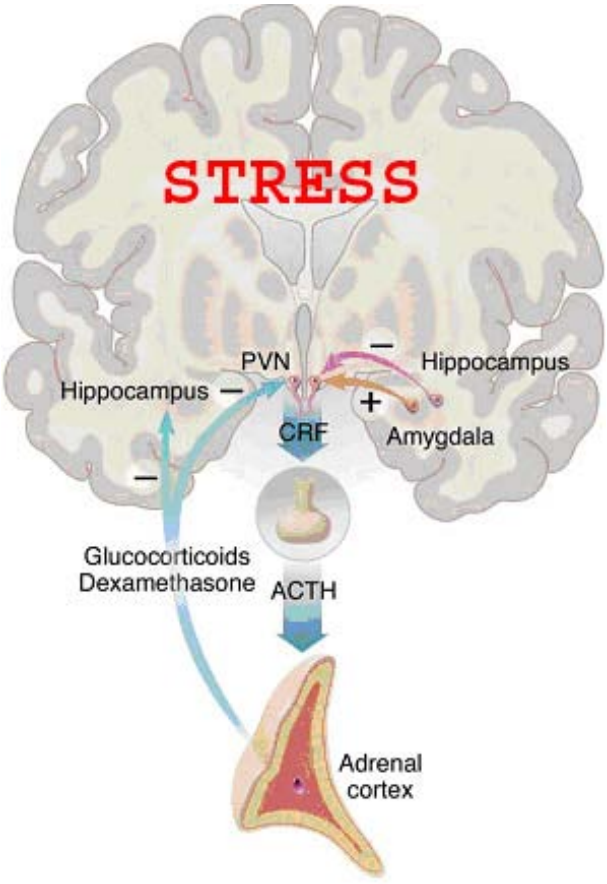

Figure 1: Schematic representation of the human HPA axis regulation under stress. PVN: Paraventricular Nucleus, CRF: Corticotropine-Releasing Factor and ACTH: Adrenocorticotropine Hormone (modified from Nestler et al. 2002).

As a second causative source, inflammatory processes have recently been suggested as inducers of Depression. For example, Interferon-alpha treatment in patient with Hepatitis C infection can result in symptoms consistent with those found in MD, such as anhedonia or fatigue (Loftis and Hauser 2004). This led to the cytokine hypothesis of Depression, which is currently under active investigation to validate its role as etiological factor in Depression (Dunn et al. 2005; Hayley et al. 2005).

1.3. Neuroanatomical circuitry of Depression

Figure 1 depicts a central role of the hypothalamus, amygdala and hippocampus in the stress experience. Although many brain areas, nuclei or circuits are involved in Depression, two main circuitries are centrally implicated: the limbic-thalamic-cortical and the limbic-cortical-striatal-pallidal-thalamic circuits (Drevets 2001). Several imaging methodologies such as Positron Emission Tomography (PET) or measurement of Cerebral Blood Flow (CBF) via Magnetic Resonance Imaging (MRI) have implied structures belonging to those two circuits in the pathogenesis of MD. In MD, an increase of cerebral metabolism was found in the amygdala, orbital cortex and medial thalamus, whereas the dorsomedial/dorsal anterolateral Prefontal Cortex (PFC) and anterior cingulated cortex ventral to the genu of the corpus callosum showed decreased metabolism (Drevets 2001; Manji et al. 2001).

This implication of various brain areas with increased or decreased activation in Depression is one principal limitation regarding the development of new therapies. Two main strategies can potentially be developed; firstly, as global variations occur throughout several networks, a systemic drug approach leading to an overall stimulation of the implicated circuits may be suitable. Unfortunately, the last fifty years have shown limited efficiency of such a strategy, which involves for example increases of the intrasynaptic levels of Serotonin or Norepinephrine. The second approach would be to target and stimulate one brain area involved in those circuits, which might then stimulate, in turn, the overall circuitry itself. In this regard, the hippocampal formation is currently the most promising structure.

1.4. The Monoamine Hypothesis of Depression

The first neurobiological explanation of Depression came up in the 1950s with the discovery by serendipity of an antidepressant side effect of the tuberculostatic drug Iproniazide, a MonoAmine Oxidase Inhibitor (MAOI) (Bloch et al. 1954). Understanding the pharmacology of MAOIs has provided the formulation of the monoamine hypothesis of Depression. Basically, all further antidepressant drugs (ADs) were derived from this first discovery and result in intrasynaptic concentration increases of Norepinephrine (NE) or 5-Hydroxy-Tryptamine (5-HT or its common name Serotonin). MAOIs inhibit the conversion of 5-HT to its metabolite 5-hydroxyindolacetic acid (5-HIAA), whereas the next generations of ADs, the Tricyclic Antidepressants (TCAs), block the reuptake of both 5-HT and NE. The next generation of ADs, the Selective Serotonin Reuptake Inhibitors (SSRIs), specifically block the reuptake of 5-HT. Although ADs produce a rapid increase of 5-HT and NE, the onset of an appreciable clinical effect usually takes at least 3 to 4 weeks. Theories that postulate long-term changes in receptor sensitivity have unsuccessfully tried to bridge this gap (Siever and Davis 1985). This delay is thus suggesting that the serotonergic and noradrenergic systems are not directly implicated for the beneficial effect of ADs in Depression.

1.5. The Hippocampal Neurogenesis Hypothesis of Depression

Although originally described by Josef Altman in 1965 (Altman and Das 1965), it has only recently been generally accepted that renewal of neurons or “neurogenesis” occurs in the adult mammalian brain. In the rodent brain, neurogenesis is restricted to two neurogenic niches: the Sub-Ventricular Zone (SVZ) of the third ventricle and the Dentate Gyrus (DG) of the Hippocampus. Thymidine analogue labelling, such as with Bromodeoxyuridine (BrdU), Iododeoxyuridine (IdU) or Chlorodeoxyuridine (CldU), is currently the prevailing method to study neurogenesis (del Rio and Soriano 1989). All mitotic cells undergoing the metaphase integrate new pools of nucleotides when replicating their Deoxyribonucleic Acid (DNA) stock. Once incorporated into the DNA, thymidine analogues can be specifically detected in postmitotic cells using immunohistochemistry.

Hippocampal neurogenesis occurs in two main steps (Fig. 2): neuronal precursors proliferate in the Sub-Granular Zone Layer (SGZ), migrate and integrate into the

neuronal network of Granule Cell Layer (GCL), where they become fully functional (Alvarez-Buylla and Lim 2004; Zhao et al. 2008). Neurogenesis is regarded as an essential process of structural plasticity for hippocampal-dependent function, such as acquiring and recovery of hippocampal-dependent memory (Ehninger and Kempermann 2008).

In light of this, it is intriguing that patients with MD exhibit prominent deficits in explicit memory, a cognitive function well established to depend on hippocampal function (Squire et al. 2004). Additionally, structural imaging has demonstrated a decrease in hippocampal volume in patients with MD (MacQueen et al. 2003). Finally, the strongest evidence leading to the implication of neurogenesis in Depression was described by Santarelli et al. (2003), who demonstrated the requirement of hippocampal neurogenesis for the behavioural effect of ADs. Although the animal model used in this study, the Novelty-Supressed Feeding test, is not specific for Depression, further animal studies have validated the implication of neurogenesis in Depression (Duman 2004). For example, stress-induced glucocorticoid elevation was found to reduce neurogenesis in rodent (Gould et al. 1992). Additionally, all classes of ADs increase neurogenesis restricted to the hippocampus, and not the SVZ (Dranovsky and Hen 2006). Under AD treatment, the modulation of neurogenesis occurs by effects on stem/precursor cell proliferation and/or survival within a time frame matching the observed behavioural effects (Malberg et al. 2000). Nevertheless, it remains unclear whether the increase of neurogenesis is a key factor for Depression remission or a convergence of biological phenomena (Duman 2004; Vollmayr et al. 2007). All studies arguing for the neurogenesis hypothesis of Depression were performed in animal models, which may limit their relevance for the human pathology. Thus, current research must aim to decipher the biological pathways leading to neurogenesis modulation under stress, Depression and AD treatment.

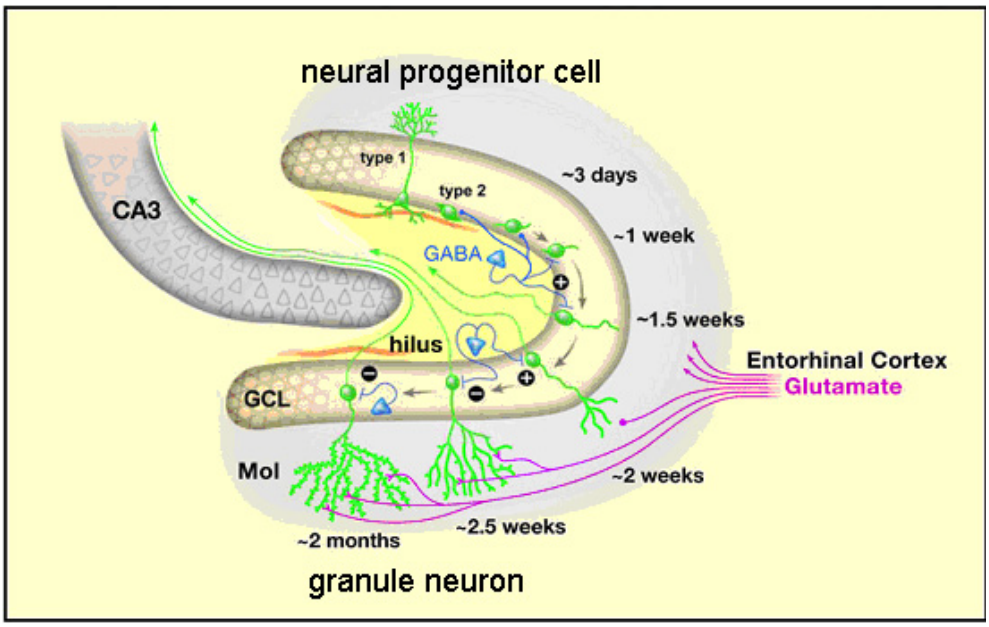

Figure 2: Neurogenesis in the hippocampal Dentate Gyrus. Type 1 and type 2 progenitor cells in the subgranular zone (SGZ) can be identified by their distinct morphologies and expression of specific molecular markers. Newborn neurons in the dentate gyrus of the hippocampus go through several stages of morphological and physiological development. Specifically, transition from excitatory Gamma-Amino Butyric Acid: GABA (blue) to inhibitory GABA and excitatory glutamate inputs to newborn neurons occurs during the third week after cell birth, concomitant with the growth of dendritic spines. Progenitor cells in the dentate gyrus are influenced by local astrocytes (not shown) and by the vasculature (red). Abbreviations are as follows: GCL, granule cell layer; Mol, molecular layer (modified from Zhao et al. 2008).

1.6. The Neurotrophin Hypothesis of Depression and the Brain-Derived Neurotrophic Factor (BDNF)

The neurotrophin hypothesis of Depression is currently under active investigation, since it may build a bridge between the monoamine and the neurogenesis explanations of Depression.

Neurotrophins are a group of small secreted proteins, which have a central role in nervous system development and adult neuronal proliferation, differentiation, migration, survival and plasticity. Four neurotrophins have been already documented: the Nerve Growth Factor (NGF), the Brain-Derived Neurotrophic Factor (BDNF), the Neurotrophin-

3 (NT-3) and the Neurotrophin-4/5 (NT-4/5). NGF plays a central role in nervous system development, whereas BDNF acts mainly in adulthood.

BDNF is a 27-kiloDalton (kDa) protein and, like all neurotrophins, is secreted as a pro-form, which is then further cleaved to generate mature BDNF of about 13 kDa. BDNF forms stable homodimers with high-affinity for the Tyrosine kinase B (TrkB) receptors. Homo-dimerization of TrkB and subsequent auto-phosphorylation of tyrosine residues in the kinase domain lead to activation of intracellular pathways involved in cell survival, proliferation, differentiation, synaptic transmission and hippocampal long-term potentiation (LTP) (Blum and Konnerth 2005). In addition to these rapid direct effects, activation of the TrkB receptor results in a long-term activation of cellular plasticity. One central component involved is the cAMP response element-binding protein (CREB), which modulates gene expression. CREB is currently regarded as the downstream effector of the BDNF pathway implicated in Depression (Gass and Riva 2007). Although neurotrophins act locally after binding to their receptor and internalization, they are also retrogradely transported and then might act in a long-distance manner (Magby et al. 2006).

Patients with MD show serum BDNF changes, which are correlated with the development of depressive episodes and with remission under AD treatment (Angelucci et al. 2005). In addition, a polymorphism in the *bdnf* coding region, which produces BDNF with either valine or methionine in position 66 of the pro-domain (Val66Met), attenuates the regulated activity-dependent release of BDNF and has been associated with mood disorder (Neves-Pereira et al. 2002).

Accordingly, animal studies revealed decreases in BDNF mRNA and protein levels in the hippocampus after chronic corticosterone injections (Jacobsen and Mork 2006). Several lines of evidence also suggest that expression of BDNF may be a downstream target of a variety of antidepressant treatments (Russo-Neustadt et al. 2004). Interestingly, administration of ADs increases the expression of BDNF mRNA in limbic structures in response to chronic, but not acute AD treatment (Nibuya et al. 1995), which is consistent with the time course typically required for the therapeutic action of antidepressants.

Previous studies using animal models of Depression addressed the antidepressant effects of BDNF administration. Central administration of BDNF was found to produce antidepressant-like activity (Siuciak et al. 1997). It has also been demonstrated that a single bilateral infusion of BDNF into the dentate gyrus of the hippocampus produced

antidepressant effects (Shirayama et al. 2002), stimulated spatial memory and decreased anxiety-like behaviour (Cirulli et al. 2004). More recent findings suggest that BDNF may have an antidepressant effect by stimulating neurogenesis and neuroplasticity in the hippocampus (Sairanen et al. 2005, 2007), although the decrease in BDNF itself may not have a causative role in leading to depression-like behaviour (Martinowich et al. 2007). Consistent with the hippocampal neuroplasticity or neurogenesis hypothesis of Depression, several lines of evidence suggest that BDNF effects on reversal of Depression phenotypes are restricted to the hippocampal formation (Eisch et al. 2003; Govindarajan et al. 2006). BDNF is thus currently the most suitable molecular target in Depression and its specific over- expression in the hippocampus is regarded as a promising approach to repair the impaired circuitry in Depression.

2. Stem cell therapy and cell-based BDNF gene therapy in the hippocampus

2.1. Rationale for region-targeted stem cell therapy and cell-based gene transfer in Depression

As described above, hippocampal plasticity is currently well accepted as a central process implicated in the development and recovery from Depression (Dranovsky and Hen 2006; Warner-Schmidt and Duman 2006; Zhao et al. 2008). A therapeutical strategy, which specifically targets this process, might be a promising alternative to current AD treatments. Indeed, current treatments present several side effects, presumably due to their influence on other physiological processes, and show high rates of symptom resurgence after treatment cessation. Given that no current drugs act strictly on a particular brain area and/or function, gene therapy targeted to the hippocampus might be a suitable alternative approach to treat Depression. There are many strategies to express a specific gene in a specific brain area. The first one consists of *in situ* injection of a viral or non-viral vector. This approach is unfortunately not guaranteeing specific expression in the targeted area and does not allow for control of ectopic gene expression. The second approach consists of integrating the gene of interest into a specific cell type before transplantation into the target structure. The

candidate cell type for autologous transplantation should be easy to isolate, to modify *ex vivo*, to propagate and to be integrated into the target structure.

2.2. Mesenchymal Stromal Cells

Mesenchymal Stromal Cells (MSC) contain promising adult pluripotent stem/progenitor cell populations, which can be used for clinical applications (Giordano et al. 2007). MSC are able to differentiate into multiple mesoderm-type cell lineages (Baksh et al. 2004) and non-mesoderm-type lineages (Sanchez-Ramos et al. 2002). As opposed to the other Bone Marrow (BM) resident cells, they are easy to isolate by their property of selective adherence to culture dishes. Finally, as adult stem cells, MSC present a powerful alternative to embryonic stem cells for clinical applications, because there are less ethical concerns. In addition to their endogenous “stem” properties for cell-based gene therapy, several lines of evidence suggest that MSC secrete a number of soluble factors, which can stimulate the surrounding microenvironment after local transplantation (Phinney and Prockop 2007). According to this hypothesis, it has been observed that local MSC implantation into the hippocampus promotes neurogenesis (Munoz et al. 2005), neuronal network formation (Bae et al. 2007), and ameliorates memory acquisition (Wu et al. 2007). Furthermore, adult hippocampal-derived soluble factors were shown to induce a neuronal-like phenotype in MSC (Rivera et al. 2006). It is thus of interest to assess whether paracrine properties of MSC might also drive antidepressant effects after hippocampal transplantation.

Despite extensive research related to MSC integration and migration after transplantation in various disease models (Chen et al. 2001; Bae et al. 2007; Wu et al. 2008) and in neonatal brain (Kopen et al. 1999; Phinney et al. 2006), little is known about the integration of MSC into the normal adult brain. For instance, recent studies showed low integration rates (Phinney et al. 2006) or entire graft loss due to host rejection after allogeneic MSC transplantation in normal adult brain including hippocampus (Coyne et al. 2006). It is thus of relevance to better characterize graft stability after allogeneic transplantation in the adult brain, particularly with regard to human clinical applications. On the other hand, transplantation with low integration or subsequent graft clearance might also limit the amount and duration of transgene expression when only transient modifications are needed.

2.3. MSC-mediated BDNF gene transfer

As described above, BDNF is currently the most promising molecular target for clinical application in Depression and the next challenge is to translate this improved understanding into better therapeutic strategies. Previous experiments aimed at increasing BDNF levels in the hippocampus were performed by local injection of BDNF (Shirayama et al. 2002; Cirulli et al. 2004), which is not ideal for human gene therapy. A BDNF gene therapy with targeted expression in the hippocampus by means of cell transplantation might be better suited.

Recently, BDNF gene therapy using MSC has been successfully performed in rat models of brain injury (Kurozumi et al. 2004, 2005). Implantation of MSC, which express BDNF after lentiviral transduction (MSC-BDNF), has shown promising regenerative properties in spinal cord injury (Lu et al. 2005). Thus, MSC-BDNF transplantation may also be a promising strategy to increase hippocampal neuroplasticity in pathologies such as Depression.

2.4. Intrahippocampal transplantation of MSC-expressing BDNF

In rodents, behavioural studies after selective lesioning of the hippocampal formation have shown a functional segregation along its dorso-ventral axis (Moser et al. 1993; Moser and Moser 1998; Kjelstrup et al. 2002; Bannerman et al. 2004; Pothuizen et al. 2004). In those studies, the ventral part was found to be primarily involved in emotion processing (Kjelstrup et al. 2002), presumably due to its preferential connectivity with the amygdala formation (Witter et al. 2000), whereas the dorsal part was found to be more involved in spatial working memory (Pothuizen et al. 2004) with a preferential connectivity with the PFC (Witter et al. 2000). Interestingly, neurogenesis occurs all along this axis and has been implicated in spatial memory as well as in Depression. Depression presents a combination of symptoms that might be seen as a global hippocampal dysfunction of memory and emotion, therefore transplantation into the middle of the hippocampus seems to be best suited for global stimulation of hippocampal structural and functional plasticity (Fig. 3).

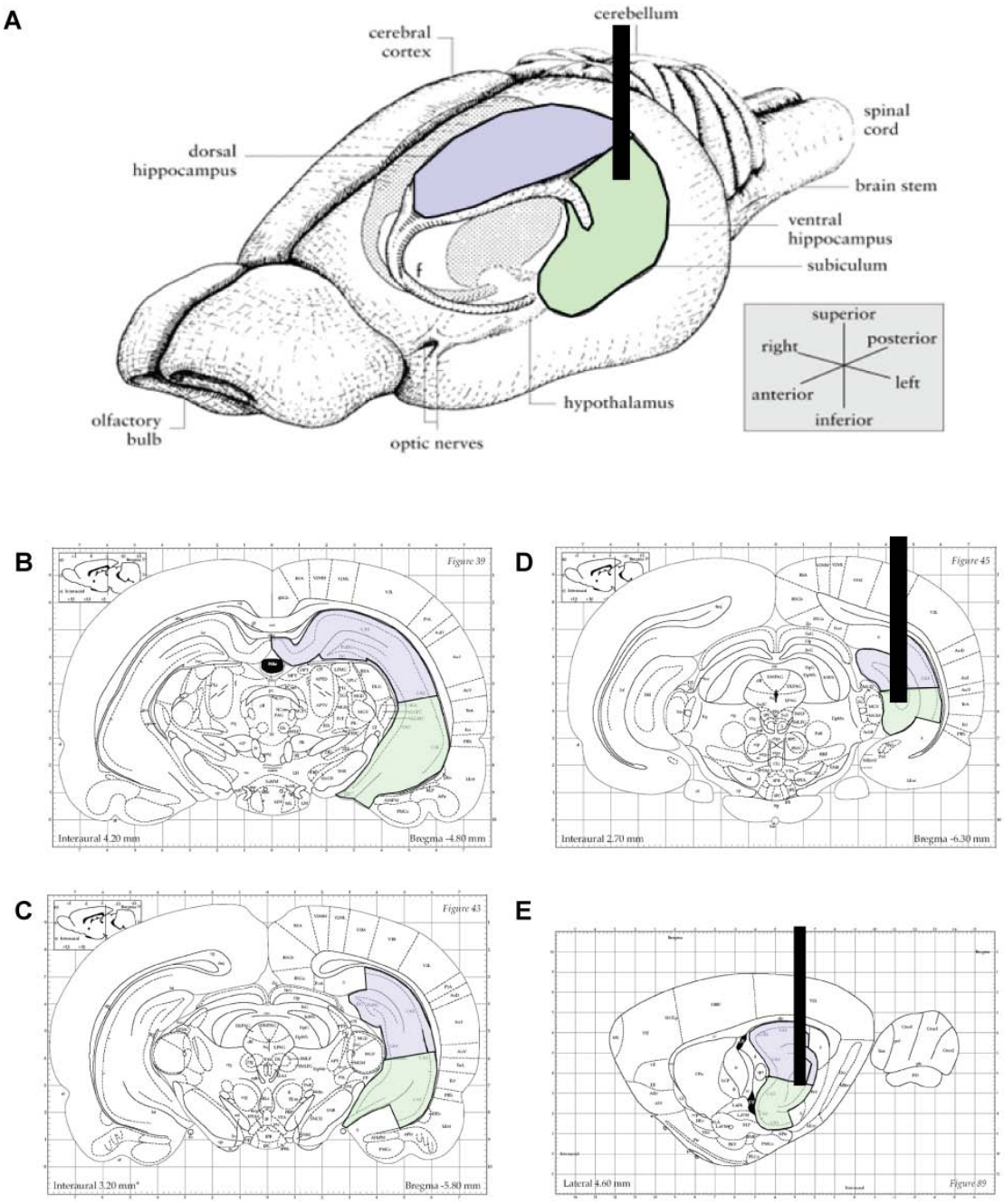

Figure 3: Functional segregation between ventral/dorsal hippocampus and localization of the site for MSC transplantation. Blue: Dorsal Hippocampus. Green: Ventral Hippocampus. Black line represents the needle trajectory targeting the middle of the hippocampus. **A**: Global view of the hippocampal formation in the rat. **B to D**: Coronal sections of the hippocampus along the anterior-posterior axis. **E**: Sagital view with target site for MSC transplantation (modified from Paxinos and Watson).

3. MRI *in vivo* cell tracking after transplantation

3.1. Rationale for non-invasive *in vivo* MRI cell tracking

In cell-based therapies, the localization and fate of the grafted cells had so far to be determined by the analysis of brain sections postmortem. Conventional methods for tracking cells include labelling with BrdU or expression of reporter genes, such as β-Galactosidase (lacZ) and the Green Fluorescent Protein (GFP). Unfortunately, these methods do not yield conclusive data on the time course of engraftment, the migration pattern of the transplanted cells within a particular host organism, and the volumetric distribution within this structure. In contrast, labelling of cells with paramagnetic contrast agents and the subsequent application of high resolution Magnetic Resonance Imaging (MRI) provide a non-invasive method to study the fate of transplanted cells *in vivo*, and to extrapolate the area of gene expression within the tissue.

3.2. Contrast agents for MRI cell tracking

Paramagnetic and superparamagnetic contrast agents are molecules, which respectively influence the T1 and T2 times of the tissues and substances surrounding them. Two main types of contrast agents are currently in use. The first are the so called “T1-agents” composed of gadolinium (Gd (III)), which result in a hyperintense contrast in the T1 ponderation MRI scan. The second MR contrast agents are superparamagnetic “T2-agents”, which are derived from iron oxide nanoparticles such as Fe2O3 and Fe3O4. There are divided in two main classes: Superparamagnetic Iron Oxide Particles (SPIO) or Ultra Small superParamagnetic Iron Oxide (USPIO), both resulting in hypointense contrast in T2 or T2* ponderation MRI acquisition. Although negative contrast leads to less detection sensitivity in T2 acquisition, T2 contrast agents are extensively used for the purpose of cell tracking because of their relatively small size and their biocompatible coating, which make them better tolerated and allow tracking *in situ*. Additionally, all superparamagnetic particles present the advantage of being detectable with Prussian Blue histochememistry, allowing postmortem validation of cell localization. Finally, nearly all of these molecules are already approved by the American

Food and Drug Administration (FDA) for use in a broad range of clinical applications, including *in vivo* molecular imaging.

During the last decade, gadolinium, SPIO and USPIO contrast agents have been successfully used in MRI cell tracking (Bulte and Kraitchman 2004). Recently, a new class of T2 contrast agents, the very small superparamagnetic iron oxide particles (VSOP) (Fleige et al. 2002), was also established for this purpose, with a highest detection sensitivity of 100 cells in a 17.5 Tesla Magnet (Stroh et al. 2005). VSOP are coated with monomer citrate for a total diameter of 9 nm, they are incorporated by endocytosis, then stored in the lysosomal compartment with low toxicity for the host cell (Stroh et al. 2004). As MSC labelling with several contrast agents including VSOP has been well characterized *in vitro* (Ittrich et al. 2005), this methodology has great potential for *in vivo* MSC cell tracking with MRI.

3.3. MSC tracking with MRI

The value of MRI in monitoring and tracking MSC has already been well established *in vivo* after experimental infarct in rat kidney (Bos et al. 2004; Ittrich et al. 2007), liver (Bos et al. 2004) and heart (Kraitchman et al. 2003; Amsalem et al. 2007), and postmortem analyses have successfully validated the MRI distribution of MSC. In the rodent brain, MSC tracking with MRI was performed after ischemic stroke (Jendelova et al. 2003, 2004; Sykova and Jendelova 2007) or to assess MSC tropism for malignant glioblastoma (Wu et al. 2008). In both cases, a migratory pattern was observed after implantation of SPIO-labeled MSC. To date, no one has performed *in vivo* imaging of magnetically labelled MSC transplanted into the rodent hippocampus.

4. Animal models of Depression

4.1. Validity of animal models of Depression

Animal models of Depression should be as close as possible to the DSM-IV definition of Depression (face validity). In practice, each model is dedicated to mimic one or several phenotypes of human Depression, such as hopelessness, helplessness or anhedonia (Table 1). Independent of the constitutive paradigm, each model also should be responding to AD treatment (predictive validity).

Animal models of Depression can therefore be separated in two main classes. The first class is dedicated to screen the efficacy of new compounds under stressful events by comparing the performance with known ADs. Examples of this class are the Forced Swim Test (FST), Tail Suspension Test (TST) and Novelty-Suppressed Feeding (NSF). The second class aims to reproduce the human phenotypes found in Depression and assesses the neurobiological variation in comparison with control animals. Examples of this class are the Learned Helplessness (LH), Chronic Mild Stress (CMS), Social Stress and Olfactory Bulbectomy (OB). In both approaches, the core components of these models are: stress (social or psychological), genetic selection for Depression phenotype or brain lesions (Wong and Licinio 2004). Given that each model has advantages and limitations, it is thus necessary to apply several models with different constitutive paradigms and to add a battery of additional behaviour tests in order to detect to what extent an observable variation is specific to depression-like behaviour. This second point is of great relevance for Depression research given the overlapping phenotypes with other psychiatric disorders, such as anxiety-related disorders.

4.2. The Learned Helplessness (LH) Model

The LH model was developed by Seligman and Maier (1967) and is considered to be one of the most valuable animal models of Depression (Willner 1984; Vollmayr and Henn 2001). It is used in pre-clinical pharmacological studies on the antidepressant effect of new compounds (Henkel et al. 2002).

In this model, animals are exposed to mild inescapable shock (induction of depression-like behaviour) and are subsequently tested for a deficit in acquiring an avoidance task. It shows an excellent validity concerning the etiology, symptomatology and prediction of treatment response. The cognitive component in the LH paradigm, namely the animal's impaired ability to concentrate, is a core feature of human Depression (Table 1), resulting in robust face validity. Lately, this test was modified by Vollmayr and Henn to improve the parameters for inescapable shock and active avoidance testing, which minimizes artefacts, random error and yields a reliable fraction of helpless animals, typically about 15 %, which correlates well with the human pathology (Vollmayr and Henn 2001).

Additionally, induction of LH behaviour results in several dysfunctions, which are consistent with the neurotophin hypothesis of Depression. LH animals show a strong up-regulation of corticosterone and a decreased expression of BDNF in the hippocampus and the PFC (Vollmayr et al. 2001). ADs can oppose the induction of LH behaviour, and this is accompanied by increased BDNF expression in the hippocampus (Itoh et al. 2004). Finally, local BDNF injection into the hippocampus results in decreased LH behaviour (Shirayama et al. 2002).

Nevertheless, one major limitation in the LH paradigm is its relative short-term maintenance of depression-like behaviour. The deficit in the avoidance task is reversed within two weeks after the initial inescapable shock training (Vollmayr et al. 2003). The LH model is thus more suitable for the short-term study of new antidepressant therapies, or to assess protective treatments against the development of Depression.

4.3. The Open Space Swim Test (OSST) model

The OSST (Sun and Alkon 2003, 2004) is a recently modified repeated version of the Forced Swim Test (FST), originally described in 1977 by Porsolt and coworkers (Porsolt et al. 1977 a,b). The FST is the most widely used psychological test for assessing pharmacological AD activity. Basically, when rodents are placed in a cylinder full of water without any escape possibilities, they gradually develop immobility behaviour after stopping active coping behaviour, such as climbing and swimming (Cryan et al. 2005).

In contrast to the FST, which is mostly related to the measurement of “behavioural despair”, the OSST model uses a large pool with the induced depressive-like behaviour resulting mainly from a lack of motivation or “hopelessness”, rather than a lack of physical space. One major advantage of this model is the ability to induce and maintain a depressive-like behaviour and to monitor the reversal of the induced phenotype, whereas the FST model is dedicated to screen antidepressant effects strictly. During three repeated trials, the active coping behaviour, measured as the total swim distance, decreases and the induced depressive-like behaviour is stable, long-lasting and allows for repeated measurement over several weeks when tested once a week (Sun and Alkon 2004). The OSST model of Depression is therefore more suitable than the LH model to assess the long-term effects of intrahippocampal MSC or MSC-BDNF transplantation against depression-like behaviour.

4.4. Complementary Behavioural Tests

One critical aspect in animal studies of Depression is the necessity to perform additional behavioural tests in order to validate the observed depressive-like behaviour. For example, the increase of activity in the FST may result from changes in global locomotion activity and not from ameliorated mood (Cryan et al. 2005). On the other hand, motor hyperactivity may have an influence on the frequency of shock termination in the LH model, resulting in less helpless behaviour. Additionally, anxiety and Depression are distinct pathologies, but present a high degree of comorbidity. Observations from Santarelli et al. (2003), which link neurogenesis and AD action, have been criticized on the grounds that the Novelty-Suppressed Feeding (NSF) model is more related to anxiety-like behaviour than depression-like behaviour. Finally, the hippocampal formation is also involved in spatial learning and memory in rodents (Kee et al. 2007). When dealing with targeted intrahippocampal therapy, it is therefore important to control for hippocampal function.

Consequently, for highest specificity regarding Depression, new therapies have to be assessed using a battery of behavioural models, such as the following non-exhaustive tests: the Open Field (OF) paradigm for locomotion, the Elevated plus Maze (EM) and Novelty-Suppressed Feeding (NSF) for anxiety-like behaviour, the 8-arm Radial Maze (RAM) or Morris Water Maze (MWM) for learning and memory, the Sucrose Preference Test (SPT) for anhedonic-like behaviour.

5. PhD Project Resume

5.1. Main Objective

The aim of this study was to evaluate MSC cell therapy and MSC-mediated BDNF gene therapy after bilateral intrahippocampal transplantation in two animal models of Depression (OSST and LH). For that purpose, we chose an allogeneic transplantation strategy by implanting Lewis rat MSC into Lewis rats. Using *in vivo* cell tracking with MRI and careful testing of rat behaviour and physiological variables, we aimed to reach a better understanding of the role of the hippocampus in Depression and its potential definition as clinical target. Moreover, the developed methodology might be a valuable tool to monitor gene therapy in a wider range of psychiatric diseases.

5.2. Objective 1: *In vitro* MSC modification and characterization

We aimed to label MSC with the contrast agent VSOP and to evaluate MSC integrity, the *in vitro* magnetic incorporation with nuclear magnetic resonance (NMR) spectroscopy, and finally their hypointense signal in a gel agarose phantom with MRI. We also aimed to transduce MSC with a lentiviral vector expressing BDNF and/or GFP, and to determine BDNF expression levels.

5.3. Objective 2: *In vivo* MSC tracking and distribution after intra-hippocampal transplantation

We aimed to determine with MRI the *in vivo* fate of MSC after bilateral intra-hippocampal transplantation during a time course of two months. MRI localization was to be validated with Prussian Blue histochemistry in postmortem tissue.

5.4. Objective 3: Transplantation effects on depressive-like behaviour and general behaviours

We aimed to assess the behavioural effetcs of bilateral MSC-BDNF or MSC transplantion into the hippocampus with the experimental design described in figure 5 and to compare with sham operated animals as negative control and with citalopram-treated animals as positive control.

As described above, the advantage of the OSST model lies in being able to repetitively assess changes in depressive-like behaviour during 4 weeks. This paradigm therefore allows to determine any therapeutical effects of MSC or MSC-BDNF on depressive-like behaviour.

The LH model is applied 6 weeks after transplantation. This paradigm therefore allows to determine any protective effects of MSC or MSC-BDNF on the development of depressive-like behaviour.

In parallel, three series of a behavioural test battery are performed in order to examine any causative association of the depression-like behaviour with locomotion using the Open Field scenario (OF), anxiety with the Elevated plus Maze (EM) and learning/memory with the 8-arm Radial Maze (RAM).

5.5. Objective 4: Evaluation of hippocampal structural plasticity

Based on the findings of Santarelli et al. (2003), it is assumed that hippocampal structural plasticity may be involved in antidepressant effects. We therefore aimed to assess the effects of MSC-BDNF and MSC on hippocampal structural plasticity by investigating its two principal components, cell proliferation and survival.

II. Methods

1. MSC source and culture

Isolated and characterized Lewis rat MSC at Passage 6 (P6) were purchased from the Tulane Center for Gene Therapy (New Orleans, USA). Briefly, MSC were harvested from the bone marrow of the femurs and tibias of 6 to 12-month-old Lewis rats by inserting a 21-gauge needle into the shaft of the bone and flushing it with 30 ml of 20%-MSC medium. Cells were filtered through a 70-µm nylon filter and the cells from one rat were plated into one 75 cm^2 flask. The cells were grown in 20%-MSC medium for 3 days, the medium was replaced with fresh medium, and the adherent cells were grown to 90% confluence to obtain samples here defined as P0 cells (Javazon et al. 2001).
Conforming to the protocol provided by the Tulane Center for Gene Therapy, MSC were routinely cultured at 37°C and 5% CO2 in 158 cm^2 Petri dishes containing 25 mL 20%-MSC medium. Plating was always performed at low cell density of 100 cells/cm^2. Medium was changed 3 days after plating and MSC were passage after 6 or 7 days in culture, corresponding to a maximal of 90 % confluence. For cells passage, medium was removed, plates were then washed 2 times with 10 mL warmed PBS and MSC were detached by adding 4 mL Trypsin/EDTA solution for 2 min. Enzymatic reaction was stopped by adding 4 mL warmed 20%-MSC medium. MSC suspension was then centrifuged for 10 min at 800 rpm, supernatant was removed and cells were resuspended in 8mL 20%-MSC medium. MSC suspension was then counted with Trypan Blue staining and the appropriate volume was added to new plates for a final cell density of 100 cells/cm^2.
An initial frozen vial from Tulane Center at P6 was recovered overnight in 20%-MSC medium and MSC were passaged until P9. Harvested MSC suspension was then pooled and stocked in frozen medium at -80°C. All further modifications and characterizations were performed from this homogenous stock that ensure reliability between analysis.

2. MSC lentiviral transduction, cell sorting and BDNF measurement

Lentivirus encoding enhanced GFP (LV-GFP) or human BDNF sequence followed by enhanced GFP under Internal Ribosomal Entry Site (LV-GFP-BDNF), were kindly provided by Armin Blesch (Department of Neuroscience, Center for Neural Repair, University of California, San Diego, USA). Both coding sequence are under the cytomegalovirus (CMV) enhancer and β-actin promoter.

After recovery of a frozen vial at P9 and subsequent culture for 6 days, MSC were plated in 6 well plates for 200 000 cells per well in 2 mL 20%-MSC medium. MSC were allowed to attach for 8 hours and plates were then transported in S2 cell culture facility (Neuro Wissenschaflticheș Zentrum, Berlin). Medium was removed, lentiviral suspension at 5.5 10^6 particles/mL for LV-GFP or at 3.5 10^6 particles/mL for LV-GFP-BDNF was added for multiplicities of infection (m.o.i) of 20 or 50. In order to increase transduction rate, polybrene was added in 3 mL 20%-MSC medium for a final concentration of 6 µg/mL. Plates were placed at 37°C in incubator overnight and the transducing medium was replaced with fresh 20%-MSC medium the day after. MSC were sorted for GFP expression 2 days after lentiviral transduction. For each constructs, MSC were harvested, washed 2 times in PBS and resuspended in PBS for a final volume of 500 µL. MSC were then sorted for GFP expression with the FACS cell sorter DiVa® (BD biosciences), collected in tubes containing 20%-MSC medium and furthered expanded at 100 cells/cm^2. The transduction stability was further tested 1 and 2 passages after transduction by assessing GFP expression with FACS Calibur® (BD biosciences). MSC were then expanded, harvested, pooled and frozen at -80°C to ensure cell suspension homogeneity for further studies.

3. VSOP Magnetic Labelling of MSC

VSOP labelling of MSC was performed according to the protocol of Stroh with minor modifications (Stroh et al., 2004). As general procedure, MSC were harvested and resuspended in 15 mL falcon tube for a final concentration of 1.5 10^6 cell/mL in a final volume of 1 mL 20%-MSC medium. Appropriate VSOP volume was then added. Tubes were placed in an incubator and cell suspensions were gently resuspended every 30 minutes. A control condition without VSOP was always performed in parallel. After labelling, free VSOP particles were washed out. Labelled cell suspensions were

centrifuged at 1000 rpm for 2 minutes, resupended with 2 mL PBS and this procedure was repeated 2 times. Each resulting suspension was then plated in 158 cm^2 Petri dish with 25 mL 20%-MSC medium and allowed to attach for a maximal of 12 hours in order to positively select viable adherent MSC.

For the determination of the best labelling condition, several VSOP concentrations (1.5 mM, 3 mM, 6 mM and 9 mM) and several durations of incubation (60, 90 and 120 minutes) were tested in triplicate in two independent studies. A control condition without VSOP particles was always performed. MSC viability was assessed directly after labelling with Trypan blue exclusion dye test. After overnight plating, differentiation potential, cell proliferation assays and magnetic signal determination with NMR spectroscopy were performed.

The best labelling condition, corresponding to the highest cell viability for the highest magnetic signal, was then validated, as *in vitro* prequisite, in MRI imaging with gel agarose phantom. This determined VSOP labelling procedure was then applied for all further animal transplantation studies.

4. MSC viability after VSOP labelling

As general procedure after VSOP labelling, the Trypan blue exclusion dye procedure was applied with the Neubauer chamber to assess cell viability. Basically, Trypan blue is incorporated in all dying cells after about 1 minute incubation time. Died cells are consequently detectable by their blue staining.

As routine procedure, 20 µL of Trypan blue 0.05 % was added to 20 µL cell suspension after desired cell suspension dilution. Mixed solution was added to the Neubauer chamber, which consists of 16 squares for a total volume of 3.2 µL. Cell numbers that were not comprised between 30 and 200 cells in the whole chamber were not accepted and further dilution were performed to reach this condition. Percentage of died cell were calculated as the number of cells with blue staining multiplied by cumulative factor of dilution and by 100 and divided by the total cell number.

5. Cell proliferation assay

To determine MSC proliferation properties after VSOP labelling, 1 000 VSOP labeled MSC were plated in triplicate in a 24 wells plate containing 1mL 20%-MSC medium per well. Medium was changed every 3 days. MSC were allowed to proliferate and cell number was determined with Trypan Blue 3, 5 and 7 days after seeding.

6. MSC osteogenic and adipogenic differentiation assay

Remaining differentiation potential after lentiviral transduction or VSOP labeling was determined. MSC were plated at 100 cells/cm^2 in 58 cm^2 petri dishes containing 10 mL 10%-MSC medium and were allowed to proliferate for 6 days. Differentiation media or control medium with 10%-MSC medium were then added and medium was changed every 3 days for 21 days.

Plates were then washed 3 times with PBS and MSC were post fixed with 10 mL 10 % Formalin for 20 min at room temperature. Control, adipogenic, osteogenic treated culture dishes were stained for each differentiation staining with 5 mL of respecting differentiation solutions for 10 min. Adipogenic differentiation was assessed by detecting triglyceride production with Oil red O staining solution, and Osteogenic differentiation was assessed by detecting bone with Alizarin S Red staining solution. Plates were then wash 2 times with tape water and further observed with optical microscopy.

7. Nuclear magnetic resonance (NMR) spectroscopy

After VSOP labelling and over night plating, MSC were harvested, centrifuged, counted and resupended in 2mL 20%-MSC medium for a final concentration of 0.3 10^6 Cells/ml. Cell suspensions were transferred in NMR glass tubes just before measurement ensuring homogeneous cell suspension. Each tube was then set into Bruker Minispec NMR and magnetic signal was measured in duplicate using spin echo sequence for T2 relaxation measurement. A graph is then displayed showing signal decay over time and the T2 time and standard deviation are calculated.

8. *In vitro* agarose gel phantom imaging with MRI

The most efficient VSOP labelling procedure, depending on time of incubation and VSOP concentration for a higher cell viability and lower T2 relaxation time, was then applied with agarose gel phantom MRI imaging.

Agarose gel was made by mixing 1.5 g Agarose in 100 ml TBE-Buffer and then by heating the solution in the microwave until clear consistency is obtained. The type of gel phantom in this study was a layered gel in 50 mL falcon tube.

For preparation, unlabeled or VSOP labelled MSC were keep at 4°C after labelling and further diluted in 20%-MSC medium to achieve 100, 1 000, 10 000 and 30 000 cells/100µl for VSOP-labelled MSC or 30 000 cells/100µL for unlabeled MSC. The 50 mL falcon tube was first filled with 10 mL melted agarose and then cooled at room temperature. Each cell layer consists of 100 µL cell suspension mixed with 100 µL melted agarose. The solution was mixed quickly and the entire volume was then dripped onto the top of the phantom gel to form each layer. The phantom was allowed to cool in room temperature for a couple of minutes followed by the addition of a gel interlayer of 2 mL agarose. After cooling, the second cell layer was added in the same manner. Layers order was the following: 30 000 unlabeled MSC, 30 000, 10 000, 1 000 and 100 VSOP labelled MSC.

For our experiments T2*-weighted acquisitions were performed to visualize signals belonging to VSOP labelled MSCs. MRI measurements were performed using the 7 Tesla Bruker Pharmascan® and the Para vision® computer program. Volume T2*-weighted acquisitions were obtained using a FLASH sequence with flip angle (FA) of 15°, repetition time (TR) of 18.5 ms, echo time (TE) of 6 ms, number of average (NA) of 16, and spatial resolution: 120 x 120 x 1 000 µm.

9. Animals

Following permission of local authorities, the study was carried out in accordance with the European Communities Council Directive (86/609/EEC) for care of laboratory animals and after approval of the local ethic committee (Senate of Berlin). All efforts were made to minimise animal suffering and to reduce the number of animals used. Naive male Lewis rats (Harlan-Winkelmann, Borchen, Germany; 6 weeks at arrival) were housed (2 animals per cage) in a temperature and humidity-controlled animal

facility with a 12-h light/dark cycle. Food and water were available ad libitum. All procedures and manipulation performed for the behavioural study are resumed in figure 5. In the MSC and MSC-BDNF transplanted groups only at least one correct intrahippocampal graft were keep for behavioural analysis. Animal number for each group was the following: control n= 17, citalopram n=18, MSCs n=27, MSC-BDNF n=14. Exclusion criteria for behavioural analysis are presented in the respective method description. Occasionally, animals were excluded for analysis due to experimental reasons. Adjusted animal numbers are presented for each result in the figure legends.

10. MSC intrahippocampal transplantation

MSC were labelled with 6 mM VSOP as described above and resupended at a concentration of 15 000 cells/µL in 0,9% NaCl. Cell suspension was then kept at 4°C all along the transplantation procedure. Stereotaxic operations (TSE biosystem, Bad Homburg, Germany) were carried out under anaesthesia after an intraperitoneal injection of 50 mg/kg sodium pentobarbital. The Dentate Gyrus (DG) in the middle of the hippocampus was specifically targeted with the following coordinates relative to Bregma: A/P, -6.3 mm, M/L, ±4.6 mm, and D/V, -5.5 mm (Fig. 3). Coordinates are given according to the atlas by Paxinos and Watson (1997) with the tooth bar at −3.3 mm. Injection protocol has already been described (Stroh et al. 2005), briefly, 2µL of cell suspension corresponding to 30 000 cells were transplanted in each hippocampus with a 26 Gauge 75 RN, 5 µL Hamilton syringe. Needle was inserted slowly and MSC were injected at a rate of 2 µL/min, MSC were then allowed to diffuse for 10 min before slowly retraction of the needle. For control sham and citalopram-treated animals, the same procedure was performed with 2 µL of 0,9% NaCl with only 2 min diffusion time.

11. *In vivo* cell tracking using MRI

For MRI acquisition, animals were anesthetized with 1-2% isofluran delivered via O_2 through a face mask. The breathing of isofluran treated animals was monitored using the Bio Trig® software (Bruker) and isofluran administration was adjusted to keep breathing rate around 30-50 breathing pulsation per minute (Fig. 4). Eyes were coated with regepithel® eye ointment for protection during scanning. For our experiments, T2*-weighted images were used to visualize signals belonging to VSOP labeled MSCs. MRI

measurements were performed using the 7 Tesla Bruker Pharmascan® (Fig. 4), and the Para vision® computer program. T2*-weighted images were acquired with identical geometry for comparison between images. 3D T2*-weighted acquisitions were obtained using a isotropic FLASH sequence with FA=15°, TR=15.5 ms, TE=6 ms, NA=6, and voxel spatial resolution of 167 μm^3. The field of view was adjusted to 3.5 cm for all subjects.

For the time course MSCs engraftment study, MRI was performed on day 1, 6, 20 and 50 after transplantation and animal were sacrified one day after acquisition (n=2 for each time point).

For the determination of graft localization in the behavioural study, MRI acquisitions were performed on day 50 corresponding to the last experimental day before sacrifice. To analyse the averaged signal distribution in the behavioural study, MRI acquisitions with low signal to noise were excluded. Selected MRI acquisitions (MSCs n=17, MSC-BDNF n=10) were manually co-registered with ImageJ software. Brain orientation was first adjusted with 3 axes: medial separation of the 2 brain hemispheres in horizontal and coronal view, and in sagital view with a tangential line between the top of cerebellum and top of cortex at the midline of the 2 hemispheres. A reference point was set up at the most anterior end of the recess of the inferior colliculus. Normal scans for anatomical hippocampal structure or thresholded scans for threshold-based T2* signal determination were then averaged with a home made Matlab script kindly written by Francisco Fernandez Klett. Threshold based volume rendering of T2*-weighted hypointense signal distribution were then performed using the AMIRA® program.

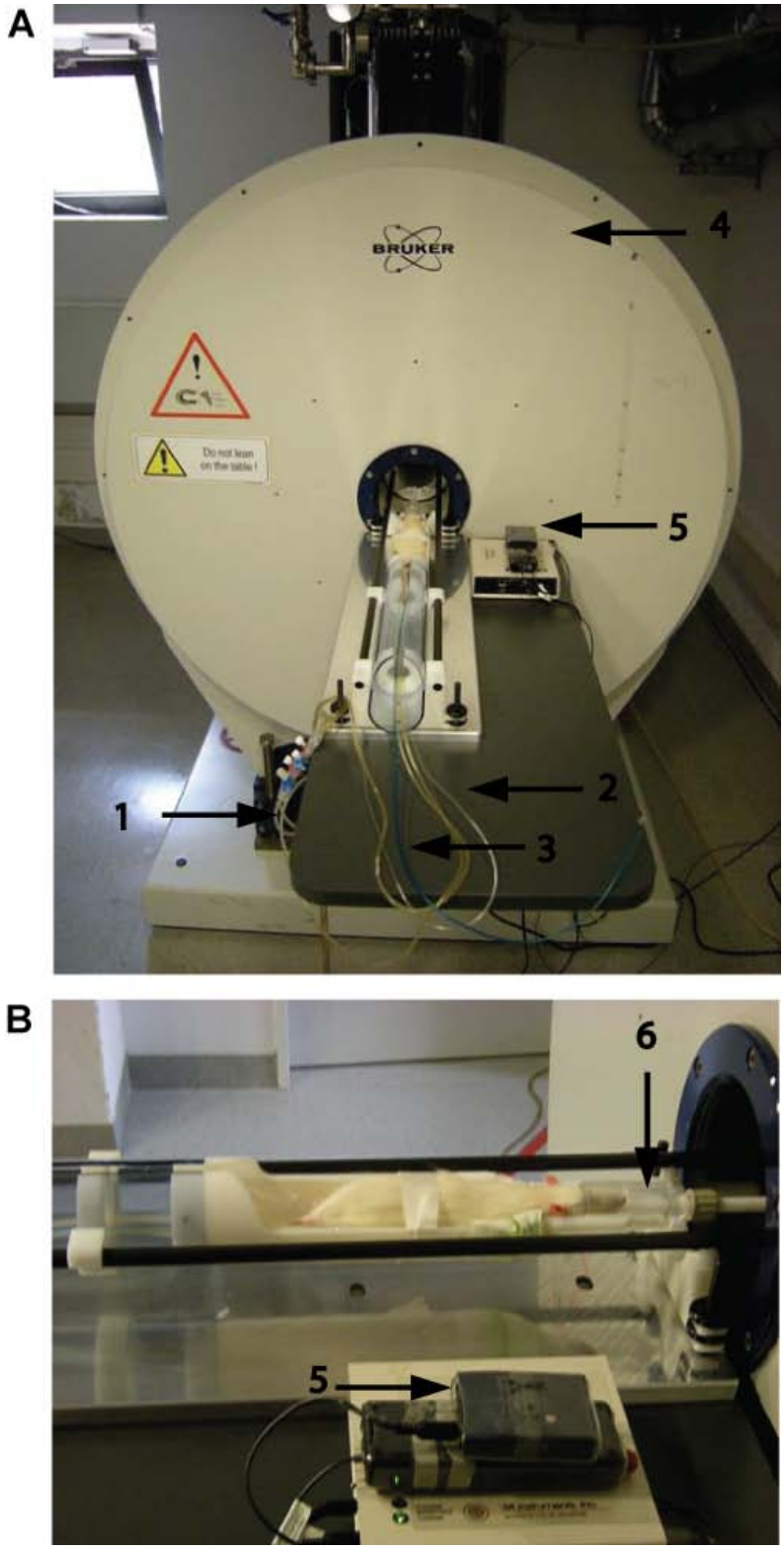

Figure 4: MRI set-up. **A**: 7 Tesla Bruker Pharmascan. **B**: Rat holder. (**1**) Heating system, (**2**) Isoflurane connection. (**3**) Breating balloon connection. (**4**) Magne Core. (**5**) Breathing monitoring digitalyseur. (**6**) Teeth holder and Breathing Mask

12. Experimental design of the behavioural study

The sequence of behavioural tests, transplantation, injections and citalopram treatment is depicted in figure 5. After arrival, animals were habituated for 2 weeks in the animal facility and were handled for 5 min/days the last 2 days of habituation. The OSST model was performed with 7 consecutives trials, with the 3 first trials (S1 to S3) given daily to induce a depressive-like behaviour and the last 4 trials (S4 to S7) given weekly to assess any antidepressant effects. Transplantation (intracranial injection: i.c.) or initiation of citalopram treatment were performed after the third OSST session determined as day 0. The LH model was performed on day 46 and 47 with an inescapable shock session (IS) followed by an active avoidance task (A). Locomotion in Open Field (OF) and anxiety with Elevated Plus Maze (EM) were performed in 3 consecutive series: before OSST (OF1, EM1), after OSST (OF2, EM2) and after LH (OF3, EM3). Memory task was performed with the 8-arm Radial Maze (RAM) after OSST with a prior food deprivation phase (day 30 to 35), a test phase (day 36 to 42), and a return to normal food regiment phase (day 43 to 45). Hippocampal cell survival and proliferation were determined respectively with injections of IdU on day 21 and BrdU on day 50. MRI acquisition was performed on day 50 one before sacrifice.

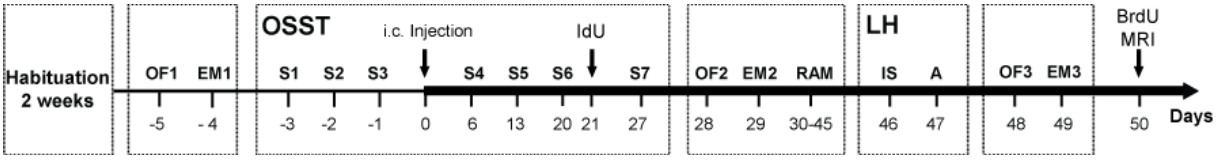

Figure 5: Experimental design and behavioural parameters measured. OSST: Open Space Swim Test, S1 to S7: successive OSST sessions, i.c: intracranial injection, LH: Learned Helplessness, IS: inescapable shock session, A: active avoidance task, OF: Open Field, EM: Elevated Plus Maze, RAM: 8-arm Radial Maze, MRI: Magnetic Resonance Imaging.

13. Citalopram treatment

Citalopram was administered via drinking water for a concentration of 30 mg/kg/day by averaging body weight for each group of 2 rats. Treatment started soon after operative day and continues until the end of experiment. The weight of rat and consumed water were measured every 3 days and citalopram concentration was then adjusted. This procedure has been already shown to produce antidepressant effect in rat and ensure lesser invasiveness in regard to chronic intraperitoneal injections (Rygula et al. 2006).

14. BrdU and IdU injection protocols

With differential immunodetection of thymidine analogues such as 5-Bromo-2'-deoxyuridin (BrdU) and 5-Iodo-2'-deoxyuridin (IdU), it is possible to address in the same animal the rate of hippocampal cell proliferation and survival (Burns and Kuan 2005). Animal were intraperitonealy (i.p.) injected with Idu at day 21, given that increase in neurogenesis appears 11 days after antidepressant treatment (Malberg et al. 2000), and cell survival was assessed 30 days later. Brdu was also injected (i.p.) at day 50 to assess cell proliferation by scarifying animal 1 day after injection (Fig. 5). Both thymidine analogues were administred with 4 consecutive injections every 3 hours for a concentration of 50 mg/kg of body weight per injection.

15. General procedures for behavioural tests

All behaviour tests were conducted with the same methodology excepted for the Learned Helplessness procedures. Experiments were carried out during the dark phase (6 pm-12 pm) corresponding to the rat period of activity. Two identical rooms of 2 m^2 were used, one for OF test, EM test, RAM test and one for OSST model. Rooms were uniformly indirectly lit with four 40 W bulbs and room environment was kept homogenous excepted for the RAM test in which spatial cues were needed. Before each trial, the platforms were cleaned with water containing 30 % ethanol. After transportation animals were allowed to habituate in their home cage to the laboratory environment for at least 15 min prior testing that reduce any novelty seeking behaviour. Behaviour monitoring was performed with a video tracking and detection software allowing calculations with animal path (VideoMot, TSE Biosystem, Bad Homburg). The Learned Helplessness test was

performed in a room from a separate laboratory during the light phase starting at 10 am. The sequence of behavioural battery tests is described in figure 5.

16. The Open Space Swim Test model

The Open Space Swim Test (OSST) model (Sun and Alkon 2003; Sun and Alkon 2004) is a recent modified repeated version of the Forced Swim Test (FST), which was originally described in 1977 by Porsolt (Porsolt et al. 1977; Porsolt et al. 1977). The FST is the most widely used psychological test for assessing pharmacological ADs activity. Basically, when rodents are placed in a cylinder full of water without any escape possibilities, they developed immobility after stopping active escape behaviour such as climbing and swimming (Cryan et al. 2005). The OSST model is performed in a large pool of 156 cm diameter with 30 cm high black stained water and water temperature keep at 25 ± 2°C (Fig. 6). Rat were gently placed in the pool and automated measurement of moved distance during 15 min is used as an index of depressive-like behaviour which is correlated to the immobility time measured in FST (Sun and Alkon 2004). As firstly described by Sun and Alkon, a first serie of three OSST sessions of 15 min swim were performed during 3 consecutive days to induce a depressive-like behaviour determined by a significant decrease of swim distance between sessions. MSC or MSC-BDNF transplantation as well as citalopram treatment initiation were performed the day after the last session. Variation of depressive-like behaviour was repeatedly assessed every 7 days for 4 weeks with the same procedure. Through consecutive swim sessions, it has occasionnaly been observed for some animals spinning swim behaviour without any arrest. Due to high swim distance, those animals were excluded from analysis with an arbitrary cut off of at least 3 spinning swim sessions in the total of the 7 sessions.

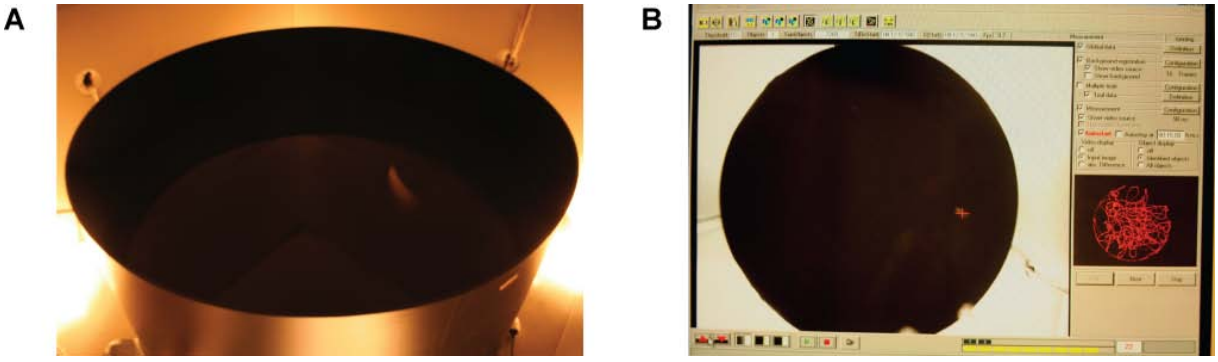

Figure 6: The Open Space Swim Test (OSST) set-up. **A**: OSST pool of 156 cm diameter dimly lit. **B**: Online automatic path monitroring with Videomot tracking software.

17. The Learned Helplessness model

17.1. Equipment

Behavioural analysis was conducted as described previously by Vollmayr and Henn (Vollmayr and Henn 2001). Accordingly, an Operant Behaviour System Mannheim Type 259900 (TSE, Saalburgstraße 157, D-61350 Bad Homburg, Germany) consisting of an operant conditioning chamber (inside dimensions: 25x30x21.5 cm), a shock generator, processing software and a compatible computer with controlling and record software were used. The conditioning chamber was equipped with a floor constructed of steel rods 6 mm in diameter and 20 mm apart from each other and steel walls allowing uninterrupted electrification. On the one side of chamber there was a protruded lever (35 x 35 mm) positioned above the steel floor and a 12 W white signal light located above the lever (Fig. 7). The ceiling of the chamber was made of plexiglass to permit observation. The shock generator delivered scrambled pulsating shocks of 0.8 mA and flexible phase-duration to floor and walls. A faulty operation was indicated by a click of the relay in the shock generator.

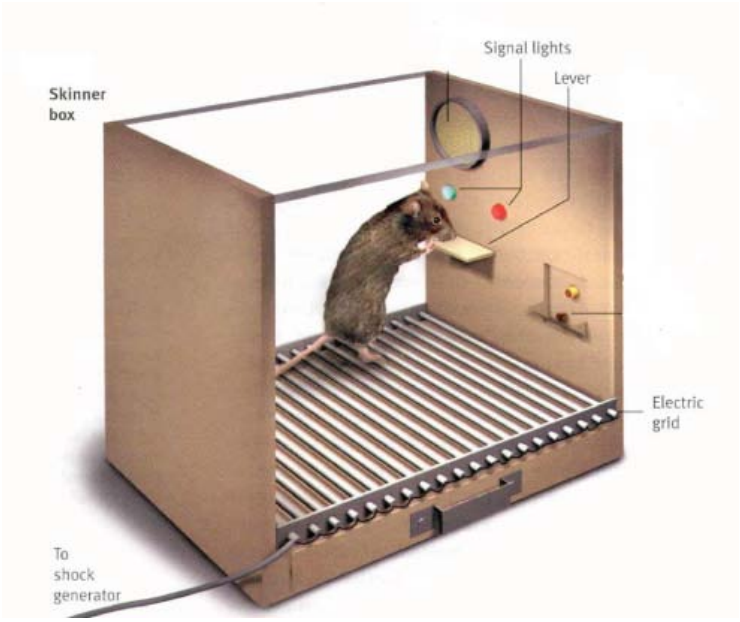

Figure 7: Operant behaviour box or “skinner box”.

17.2. Conditioning to inescapable shock

Animals were gently placed into the conditioning chamber exclusive of lever. Conditioning phase consists of a 40 min inescapable shock session (intensity: 0.8 mA, phase duration: 200 ms), with inter-shock times ranging from 5 to 15 s randomized by the computer. The total shock exposure was 20 min. After shock exposure rats were gently removed to their home cages.

17.3. Active avoidance of escapable shock

24 h after the inescapable shock session rats were gently replaced in the conditioning chamber equipped with lever and light and presented with escapable shock (intensity: 0.8 mA, phase duration: 200 ms), consisting of 15 single trials lasting for 60 s with inter-shock times of 24 s. Shocks were accompanied by a light presentation to facilitate detection of the lever and discrimination to the inescapable shock presentation. Pressing the lever by the rats stopped shock administration. Releasing the lever briefly was mandatory for being able to stop the next shock. The computer system recorded the time to terminate the shock for each animal and each trial. Data from active avoidance tests were analyzed according to the absolute parameter, i.e. the sum latency to escape shock

presentation for the 15 trials (Musty et al. 1990). Activity during test was measured with the number of lever press.

18. Open Field Test

The open-field area consisted of a 1 m^2 quadratic black platform elevated 20 cm above the floor. Each rat was placed in the centre of the Open-Field and the travelled distance was monitored for 10 min.

19. Elevated Plus Maze

The maze was elevated 70 cm above the floor and has four 50 cm x 10 cm arms. Two arms are enclosed by 22.5 cm walls and the other two arms are open with an edge of 3 mm to prevent the animals from slipping off the maze (Fig 8). Rats were placed in the open arm facing the distal end of the arm and were allowed to explore the maze for 10 min. Rat behaviour was monitored and the percentage of open arm entry performed with at least the two forepaws was determined.

Figure 8: The Elevated Plus Maze consisting of 2 closed and 2 open arms.

20. Eight-Arm Radial Maze

Following the methodology developed by Jarrard (Jarrard 1983), two types of memory processes: working and reference memory were assessed with the 8-arm Radial Maze (RAM). The test was conducted according to the protocol of (Balschun et al. 1999) with sliced modifications. Briefly the maze consisted of eight equally spaced arms (42 x 10 cm) projecting outward from a central octagonal area (50 cm across). At the distal end of each arm was located a semi-circular food cup in order to present a positive reinforcers consisting of a quarter of a peanut. The whole apparatus was directly placed on the floor. The maze was surrounded by several distinct cues (e.g. posters, doors and a brush) remaining in a constant location from trial to trial allowing spatial orientation (Fig. 9).

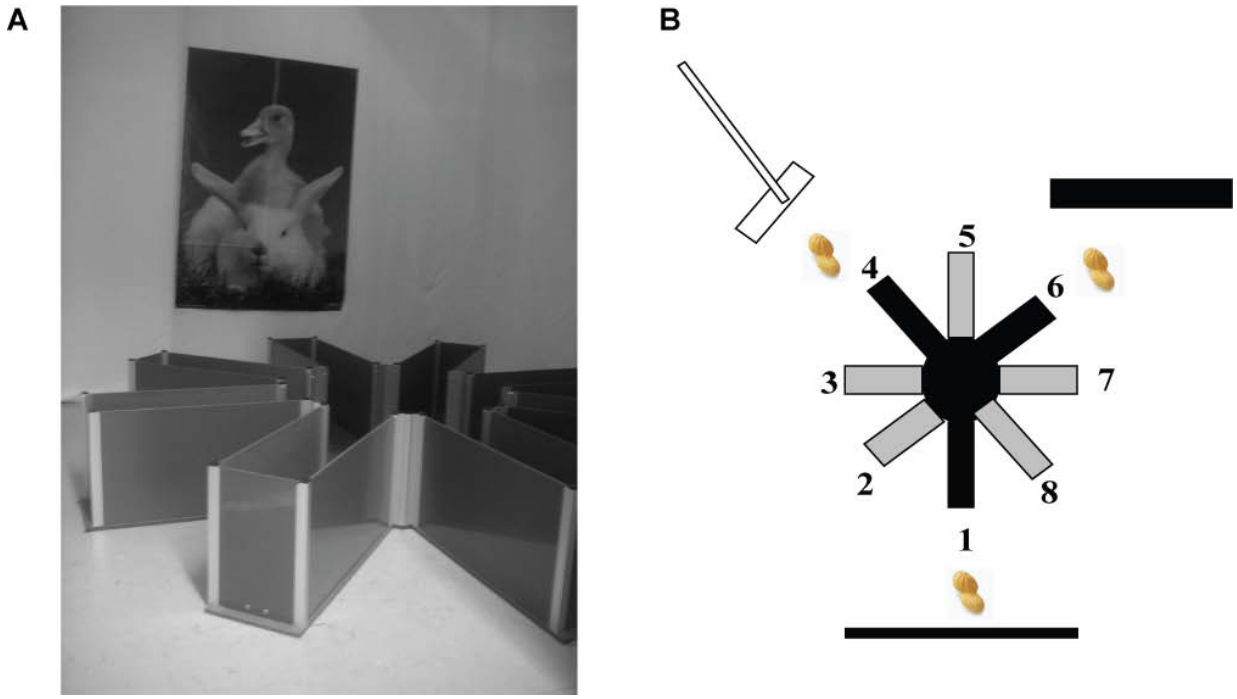

Figure 9: The 8-arm Radial Maze (RAM). **A**: Picture of RAM set-up with a poster as example of spatial clue. **B**: Schematic organisation of spatial clues (brush, poster and door) and positive reinforcers (peanut) which remain at constant locations across trials (arms 1, 4, 6).

Five days before starting the experiment and during all the duration of the test, rats were subjected to a food deprivation regimen of 21 hours per day with water ad libitum. Animal body weight was monitored daily to keep it at a maximum level of 85 % of normal body weight. The 8-arm Radial Maze procedure was performed during 8 days, with two consecutive trials per day and an interval of 2 hours between trials. Each trial started by placing the rat in the middle of the central area. During the 2 firsts days, all arms were baited, animals were habituated to the maze and train to find the reinforcers. Each trial was ended after 10 min or when all reinforcers have been eaten.
Memory tasks were performed during the last 6 days. In this second phase, only three arms were baited with constant location between trials and days. Animals were free to explore the maze and the test was completed after 10 min or when all reinforcers have been eaten. Entry into an unbaited arm was scored as Reference Memory Error (RME), and any arm re-entries were scored as Working Memory Error (WME). Data were averaged across blocks of two sessions.

21. Perfusion, postmortem brain processing

Animals were deeply anaesthetized with chloral hydrate (50 mg/kg) and transcardially perfused with 0.1 M phosphate buffer followed by 4% ice-cold paraformaldehyde (PFA). Brains were removed, postfixed in PFA overnight and subsequently transferred to 30% sucrose. Coronal sections of 30 µm thickness were cut along the entire hippocampal formation on microtom with dry ice. Sections were kept in cryoprotective solution (glycerol, ethylene glycol, 0.1 M phosphate buffer) in a 48 plate format.

22. Histology, Histochemistry, Immunohistochemistry

Prussian Blue staining was performed as free floating in equal parts 4% w/v potassium ferrocyanide solution and 2 M hydrochloric acid for 10 minutes. Counter staining was performed using eosin.
For immunohistochemistry, slices were firstly blocked with 10 % Normal Donkey Serum (NDS) and 0,3 % Triton-X-100 for 2 hour at room temperature. Slices were then incubated overnight at 4°C with primary antibody in 3 % NDS and 0.3 % Triton-X-100. Secondary antibody was added for 2 hours at room temperature. For BrdU and IdU immunodetection, every sixth slices along the entire hippocampus were processed as

free-floating sections. Before immunodetection, slices were treated to denaturate DNA strand with 2 N HCl for 20 min at 37°C. Neutralisation was then performed with 0.1 M Borate Buffer for 12 min at room temperature and slices were subsequently processed as described above.

Determination of cell proliferation was performed by counting BrdU positive cell clusters in the Sugranular Zone (SGZ) as determined as group of at least one single cell separated from other clusters with a distance of at least two nuclei. Cell survival was determined by counting IdU positive cells presenting round nuclei with homogenous fluorescent signal either in the SGZ or in the Granular Cell Layer (GCL). Quantification was performed by averaging in each group the number of respecting BrdU and IdU counts per slice (total analyzed slices per group: control n= 336 for 10 animals, citalopram n= 255 for 8 animals, MSC n= 312 for 9 animals, MSC-BDNF n= 313 for 9 animals).

23. Statistics

Before analysis, normal distribution and normality of variance were controlled respectively with Shapiro-Wilk test and Levene test. For OSST and RAM analysis, differences between groups were determined with repeated measurement of Analysis of Variance (RM-ANOVA). OF and EM variations were analyzed with 1 way ANOVA. Group comparison to control was then analyzed by Dunnett post hoc t-test or with Tamhane's T2 correction of variance when appropriate. For Learned Helplessness as well as for hippocampal cell proliferation and survival, statistical analysis was performed with non-parametric Mann-Whitney test with subsequent Bonferroni correction when appropriate. A probability level $p \leq 0.05$ was considered to be statistically significant.

III. Results

1. *In vitro* MSC characterization after VSOP labelling

MSC were incubated with VSOP at final concentrations of 0, 3, 6, 9 mM and were directly tested for cell viability with Trypan Blue. T2 time was assessed with relaxometry, proliferation and differentiation properties of VSOP-labelled MSC were analyzed after seeding.

1.1. Relaxometry and cell survival

T2 relaxation time from unlabelled MSC was 1100 ± 28 ms, and reduced to 1005 ± 36, 748 ± 30 and 647 ± 9 ms for MSCs incubated with 3, 6 and 9 mM VSOP, respectively (Fig. 10A). Cell viability was maintained > 95 % when incubated with 3 and 6 mM VSOP (Fig. 10B). In contrast, labelling with 9 mM VSOP decreased cell viability to 80 %. Thus, MSC labelling was subsequently performed with a concentration of 6 mM VSOP that showed efficient magnetic labelling with sufficient maintenance of cell viability.

1.2. Cell proliferation and VSOP localization

Cell proliferation was analyzed after labelling of MSC with 6 mM VSOP. MSC cell numbers were determined 3, 5 and 7 days after seeding. VSOP labelling did not change the MSC renewal rate in comparison with 0 and 3 mM VSOP (Fig. 10C). VSOP particles were visualized with Prussian Blue staining, which revealed random cytoplasmic distribution (Fig. 10D) with homogeneous distribution during cell mitosis (data not shown). Labelling of Lewis rat MSC with VSOP is thus feasible and does not impair the *in vitro* proliferative property.

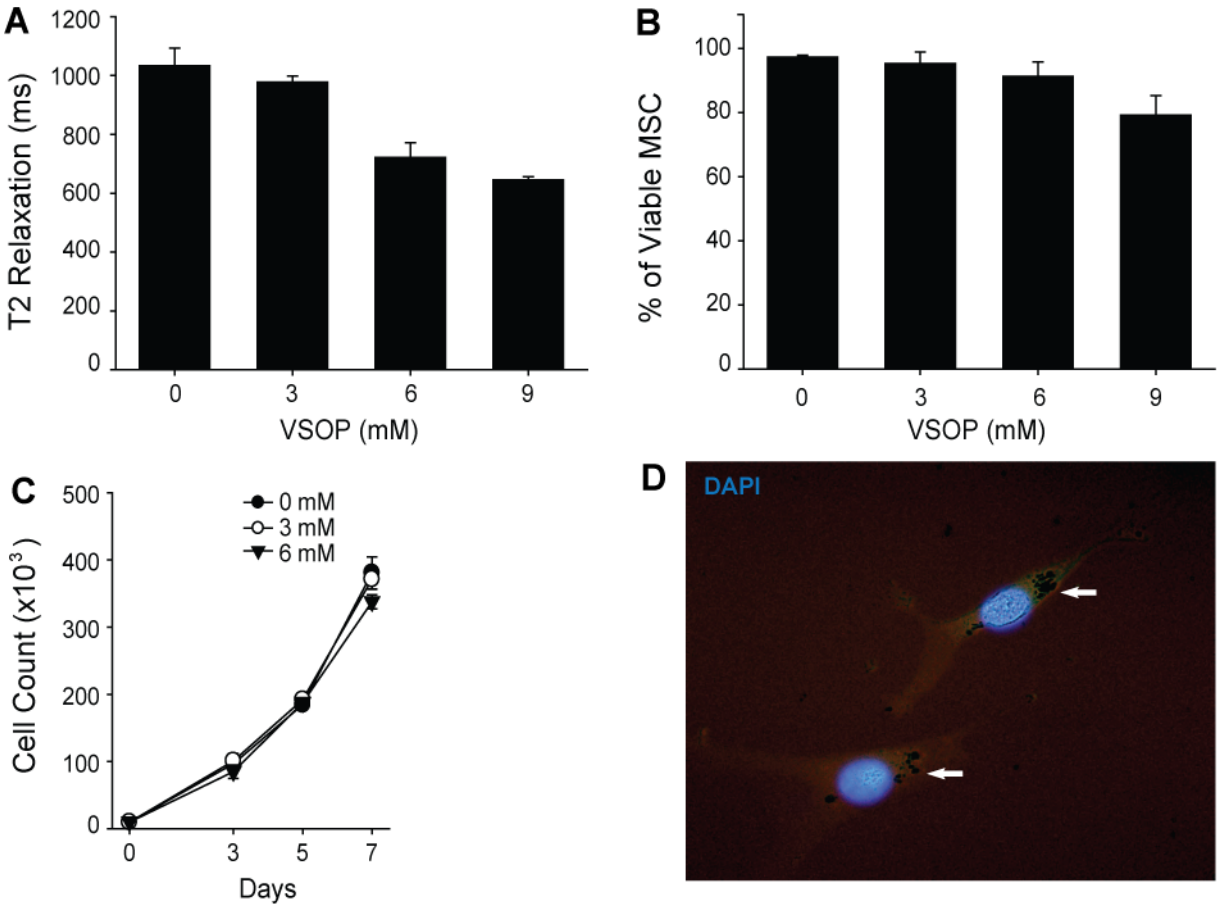

Figure 10: *In vitro* determination of the most efficient magnetic labelling condition with 0, 3, 6 and 9 mM VSOP. **A**: Magnetic signal measurement with relaxometry. **B**: Cell viability determined by Trypan Blue dye exclusion directly after VSOP labelling. **C**: Cell proliferation assessed 3, 5 and 7 days after labelling. **D**: Subcellular VSOP localization (white arrows) determined with Prussian Blue after labelling and seeding for 12 hours. Results are expressed as means ± SD.

1.3. MSC differentiation after VSOP labelling

After labelling with 6 mM VSOP, effects on the differentiation potential of MSC were assessed. Labelled MSC (Fig. 11A) were cultivated in osteogenic and adipogenic differentiation medium and subsequently stained for triglycerides and bone production, respectively. After VSOP labelling, MSC retained their ability to differentiate into adipocytes (Fig. 11B) and osteocytes (Fig. 11C). Thus, long-term maintenance of MSC properties after labelling with VSOP particles was validated.

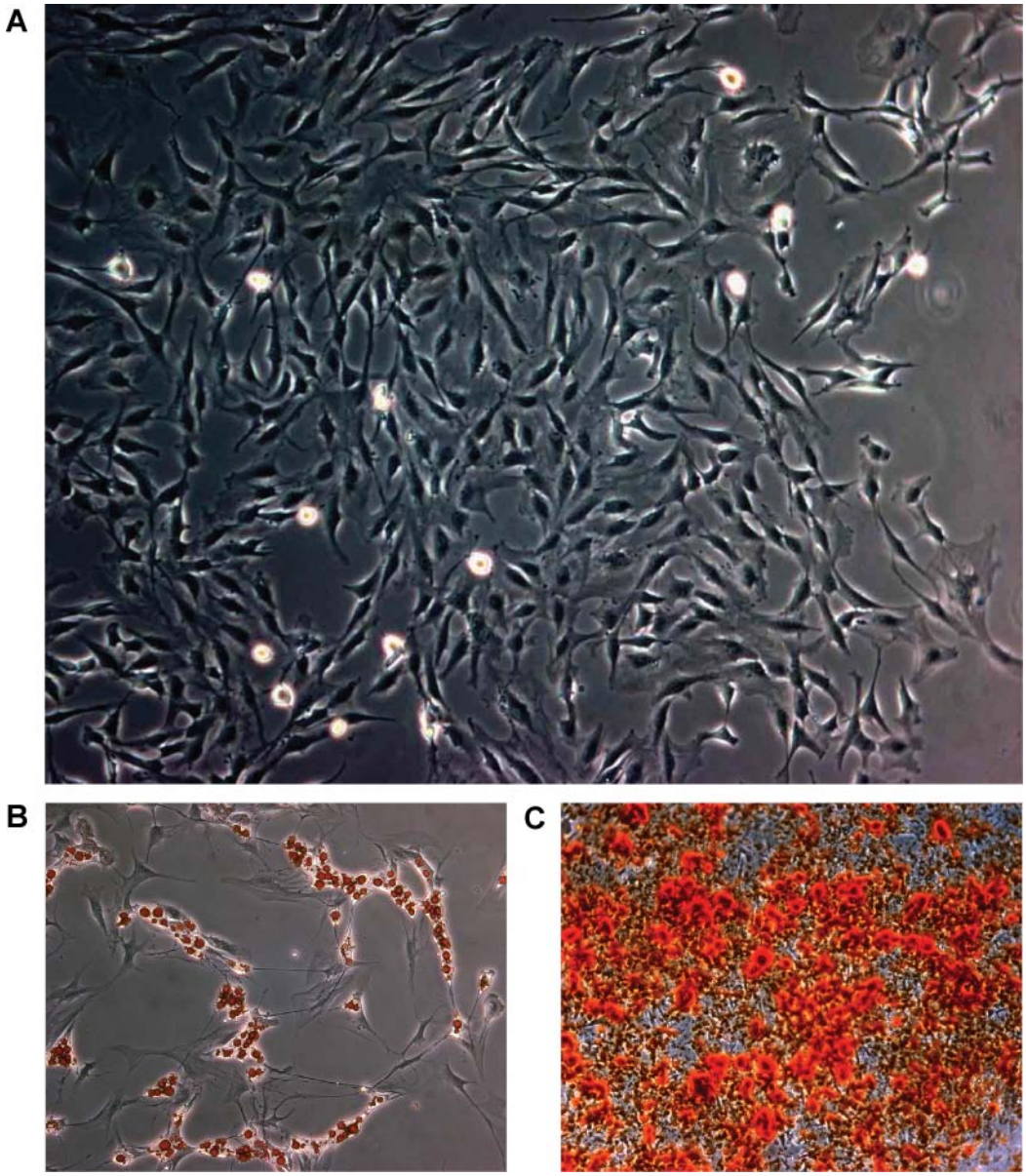

Figure 11: Maintained differentiation potential after labelling with 6 mM VSOP. **A**: Classical MSC fibroblast-like morphology under cultivation with control medium. **B**: Triglyceride production revealed by Oil red O after induction with adipogenic medium. **C**: Bone production revealed by Alizarin Red S after induction with osteogenic medium.

1.4. *In vitro* imaging of VSOP-labelled MSC with an agarose gel phantom

As a prequisite before performing *in vivo* MRI cell tracking, *in vitro* visualization of VSOP-labelled MSC with an agarose gel phantom was performed with T2* MRI ponderation. MSC labelled with 6 mM VSOP were detectable as clusters of hypointense

signal (“black spots”) for 30 000, 10 000, 1 000 and 100 seeded MSC (Fig. 12B, C, D, E, respectively). In comparison, 30 000 unlabelled MSC (Fig. 12A) as well as culture medium (Fig. 12F) showed no hypointense signal, thereby validating the effect of VSOP on T2* MRI ponderation.

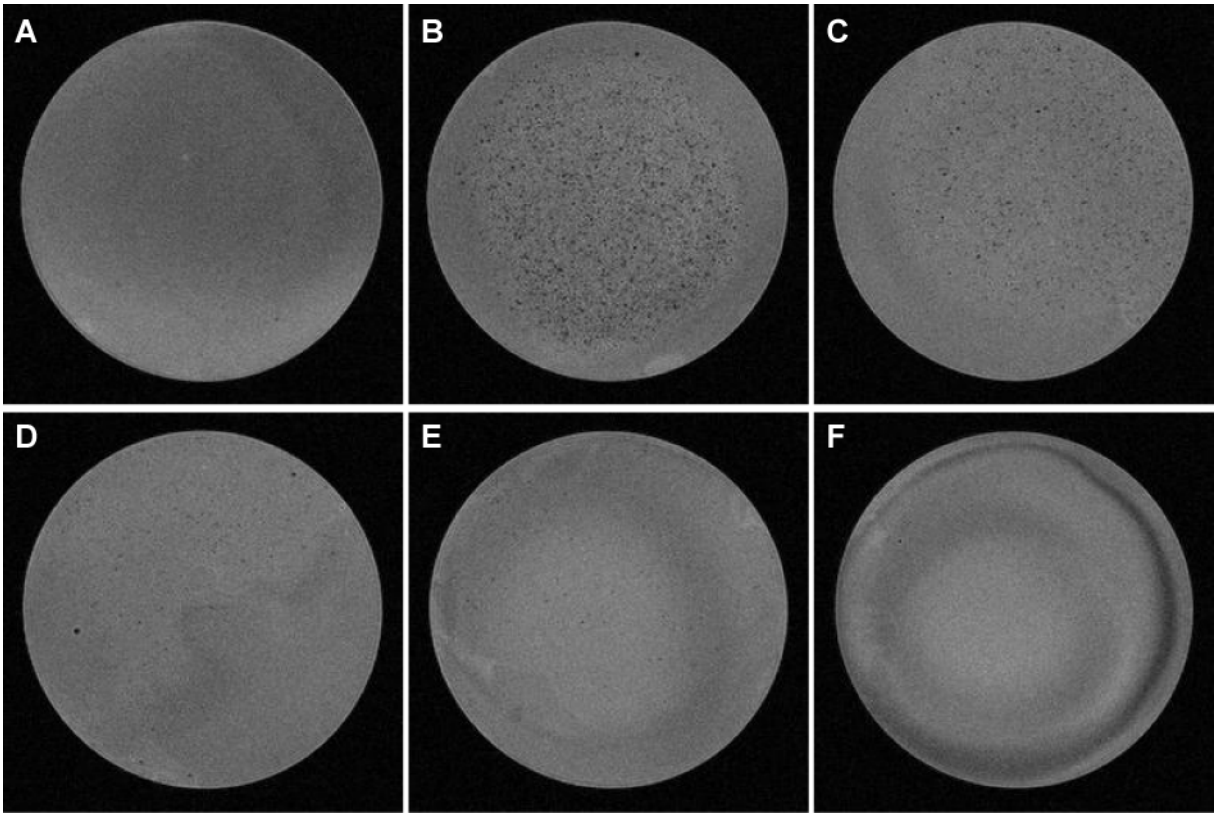

Figure 12: *In vitro* T2* MRI imaging of labelled MSC seeded in an agarose gel phantom. VSOP (6 mM)-labelled MSC are detected as hypointense “black spots”, **A**: 30 000 unlabelled MSC, **B**: 30 000 VSOP-labelled MSC, **C**: 10 000 VSOP-labelled MSC, **D**: 1 000 VSO-labelled-MSC, **E**: 100 VSOP-labelled MSC, **F**: control medium.

1.5. MRI cell tracking for long-term *in vivo* localization

After intrahippocampal transplantation of 30 000 VSOP-labelled MSC, graft localization and migratory patterns of MSC were analyzed. Serial longitudinal MRI acquisitions were performed and hypointense signal stability was determined. The MSC-VSOP graft was detectable as hypointense signal in T2* MRI ponderation at 1 day after transplantation (Fig. 13A).

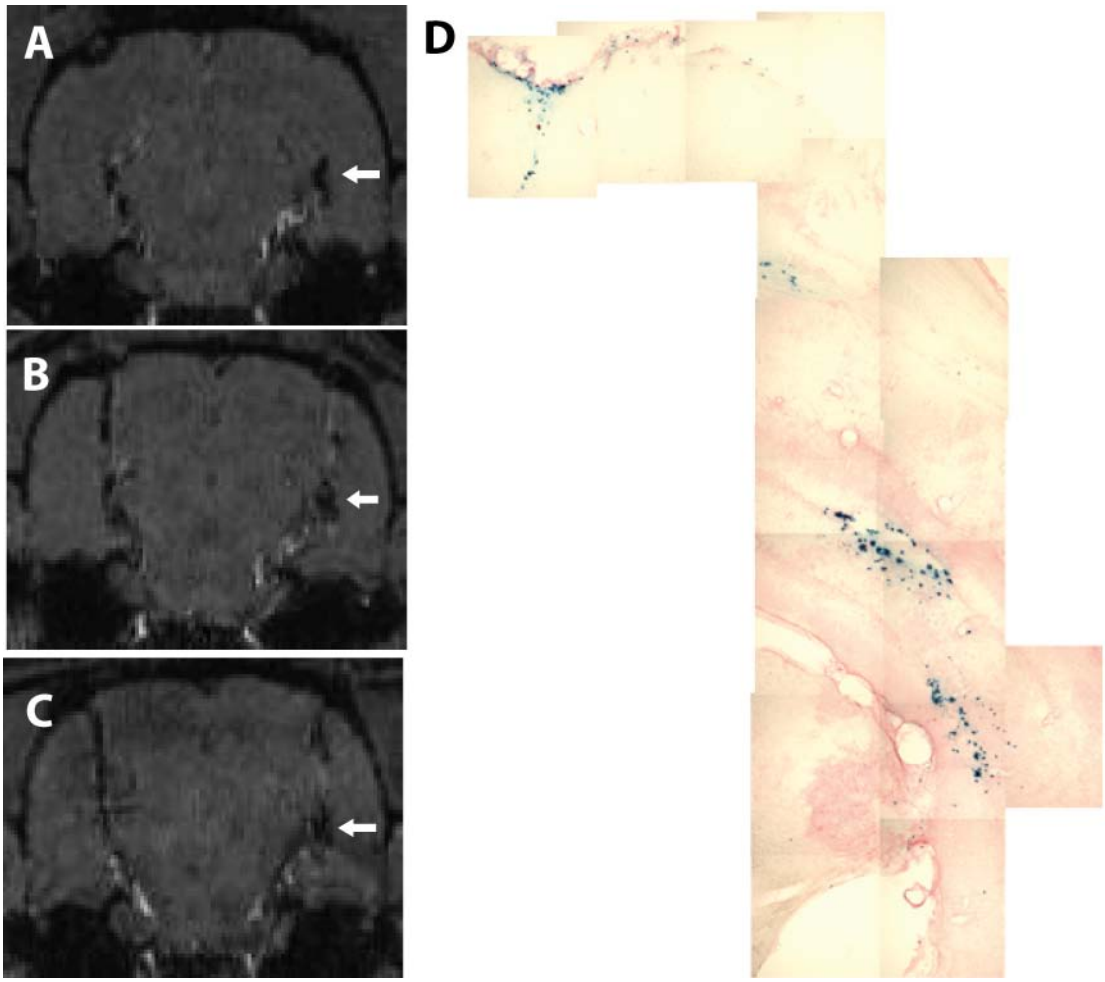

Figure 13: MRI serial acquisition of 30 000 VSOP-labelled MSC. **A**: 1 day, **B**: 20 days and **C**: 50 days after transplantation with persistent location of the hypointense signal (white arrow). **D**: VSOP detection with Prussian Blue histochemistry at 50 days after transplantation (reconstruction of a coronal section from a different animal).

Although the hypointense signal diffused slightly through sequential acquisitions, its localization remained stable for 20-50 days after transplantation without any migratory pattern (Fig. 13B and C, white arrow). Increased hypointense signal was observed all along the needle track and in the corpus callosum at 6 days after transplantation (data not shown), which most likely resulted from local haemorrhage. 50 days after transplantation, VSOP were still detectable with Prussian Blue histochemistry (Fig. 13D) and VSOP distribution in the tissue matched well with the T2* MRI distribution (data no shown).

2. MSC Transduction with GFP or BDNF-IRES-GFP lentiviral vectors

2.1. Stability of GFP expression

MSC were transduced with lentiviral vectors encoding GFP (LV-GFP) or BDNF plus GFP linked by an internal ribosomal entry site (LV-GFP-BDNF). After transduction, but before cell sorting, 47.9 % of MSC transduced with the BDNF-GFP lentivirus (MSC-BDNF) expressed GFP for a m.o.i. of 20 (Fig 14A). In comparison, 46.9 % of MSC transduced with the GFP lentivirus (MSG-GFP) expressed GFP for a m.o.i of 50 (Fig 14D).

After cell sorting and subsequent cultivation for one passage, MSC-BDNF and MSC-GFP showed a high percentage of GFP expression in 95.4 % and 89.5 % of cells, respectively (Fig. 14B and E). The long-term maintenance of GFP expression was determined two passages after the initial cell sorting. MSC-GFP and MSC-BDNF maintained the high percentage of GFP expression in 89.5 % and 95.4 %, respectively (Fig. 14C and F), indicating stable lentiviral integration and expression despite cell proliferation.

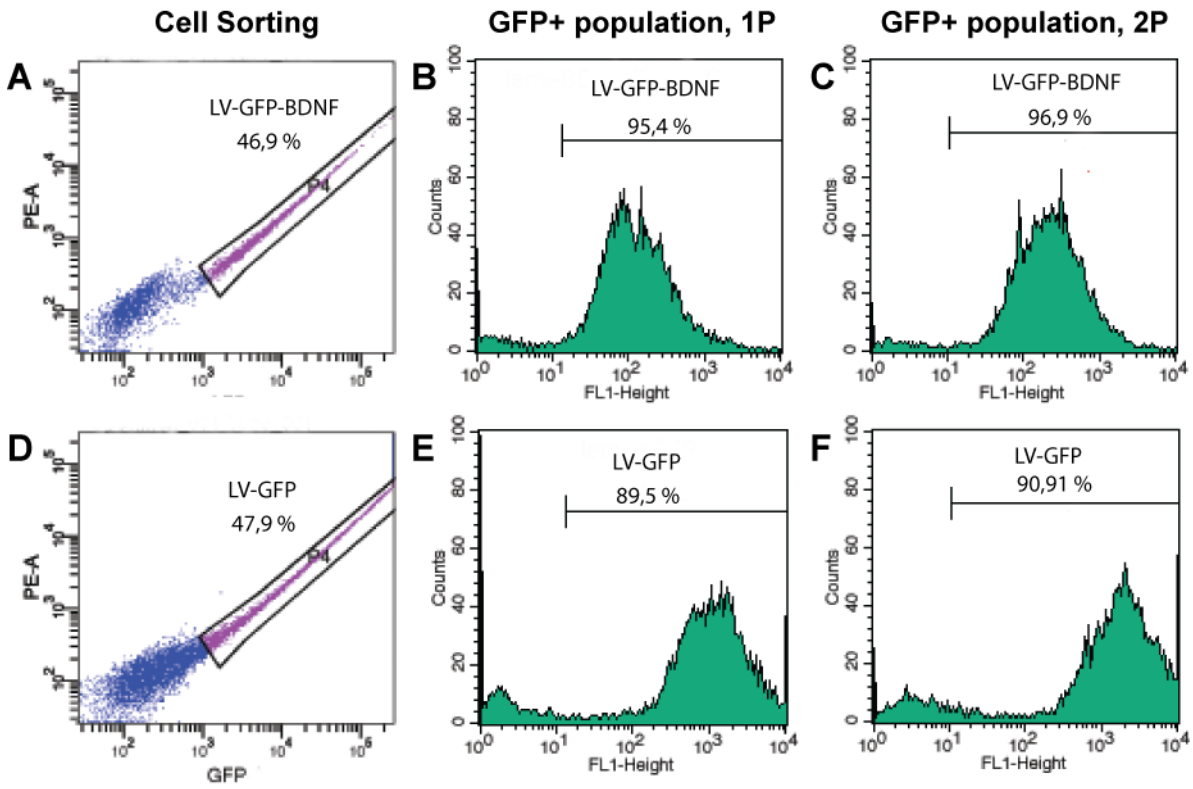

Figure 14: MSC cell sorting and long-term GFP expression after transduction with LV-GFP-BDNF (A-C) and LV-GFP (D-F). **A**, **D**: Subpopulation of GFP-expressing MSC were selected by cell sorting 2 days after lentiviral transduction. **B**, **E**: Stability of GFP expression at 1 passage after initial cell sorting. **C**, **F**: Stability of GFP expression at 2 passages after initial cell sorting.

2.2. Quantification of BDNF expression with ELISA

Detection of GFP in MSC-BDNF indicated that these cells also expressed BDNF, since BDNF and GFP were both translated from the same messenger RNA. Quantitative expression levels of BDNF were determined with ELISA. MSC-BDNF secreted 740 ± 140 pg BDNF/30 000 cells/24h (Fig. 15). In comparison, no BDNF secretion was detected in MSC-GFP supernatants.

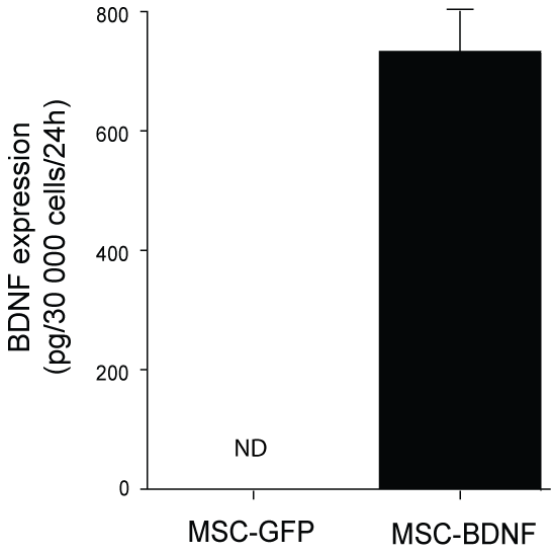

Figure 15: BDNF expression of MSC after lentiviral transduction with LV-BDNF-GFP. MSC-GFP and MSC-BDNF were seeded for 24 hours and secreted BDNF levels in the supernatatant were determined with ELISA. Results are expressed as means ± SD.

3. *In vivo* effects of MSC and MSC-BDNF transplantation on Depression like-behaviour

3.1. MRI averaged graft distribution

Stable localization of the MRI signal was used to determine the average distribution of transplanted MSC and MSC-BDNF in the behavioural experiments. Similar distribution patterns were observed after transplantation of MSC-BDNF (Fig. 16A) and MSC-GFP (Fig. 16B). The average signals were mainly found in the hippocampus, along the needle track and at the top of hippocampal formation, corresponding to corpus callosum signal spread.

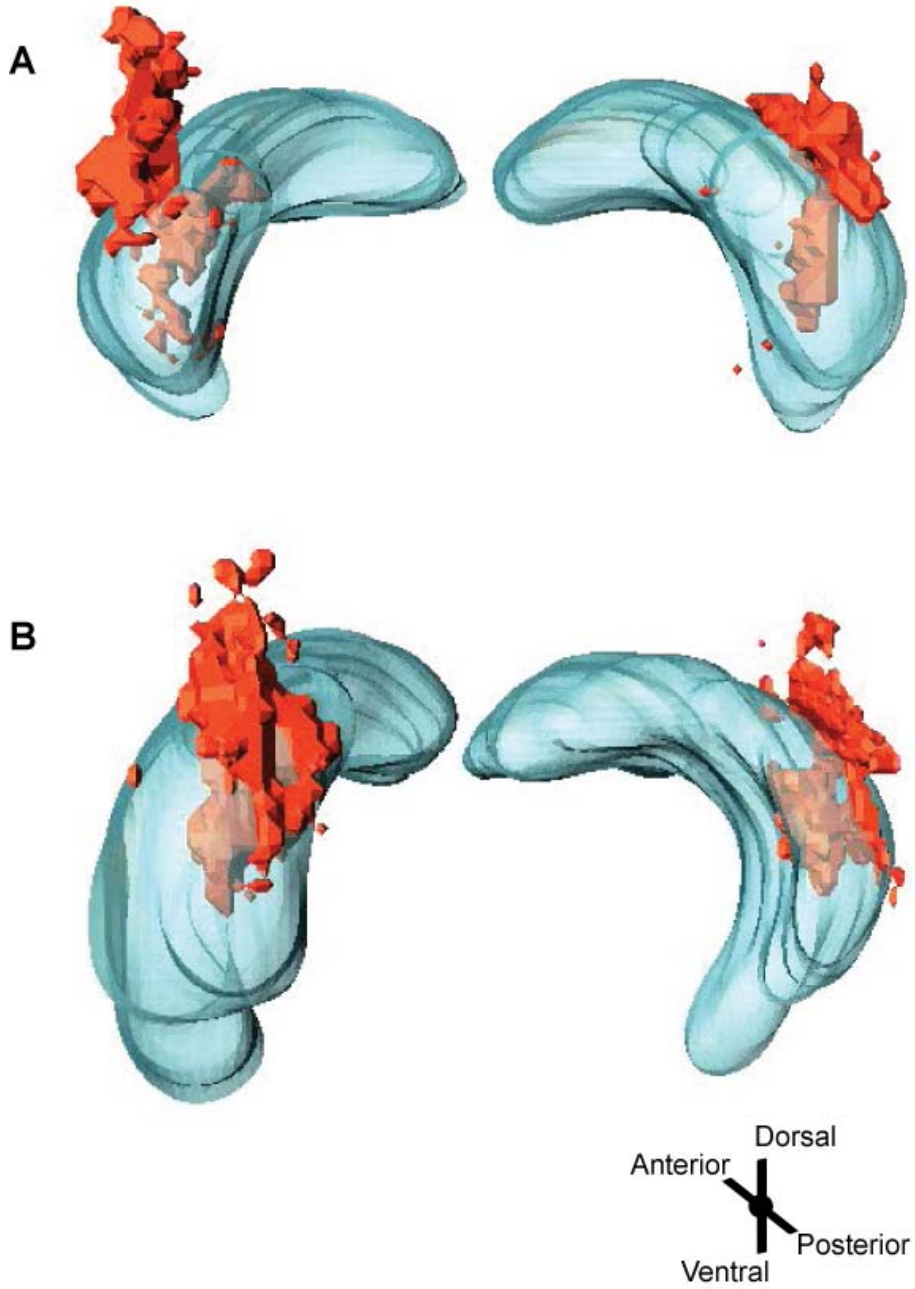

Figure 16: Averaged threshold-based volume distribution of T2* hypointense signal (orange) 50 days after transplantation of **A**: MSC-BDNF (n=10 animals) and **B**: MSC (n=17 animals) in the hippocampal formation (blue) from all animals included in the behavioural study. Perspective from the dorsal side of the hippocampal formation.

3.2. MSC and MSC-BDNF have no long-term antidepressant effects in the OSST model

Antidepressant effects of intrahippocampal MSC or MSC-BDNF transplantation were first assessed in a repeated swim stress model of Depression. For this purpose, the OSST, a modified version of the well-established FST, which was implemented for repeated trials, was used. A depressive-like behaviour was induced with 3 consecutive sessions at days -3, -2 and -1 prior to transplantation (Fig. 17). RM-ANOVA revealed a statistically significant decrease of swim distance within successive swim sessions for each of the four groups (P<0,001 for control, citalopram, MSC and MSC-BDNF groups, -1 versus -3 days), where between-group comparisons showed no statistical difference.

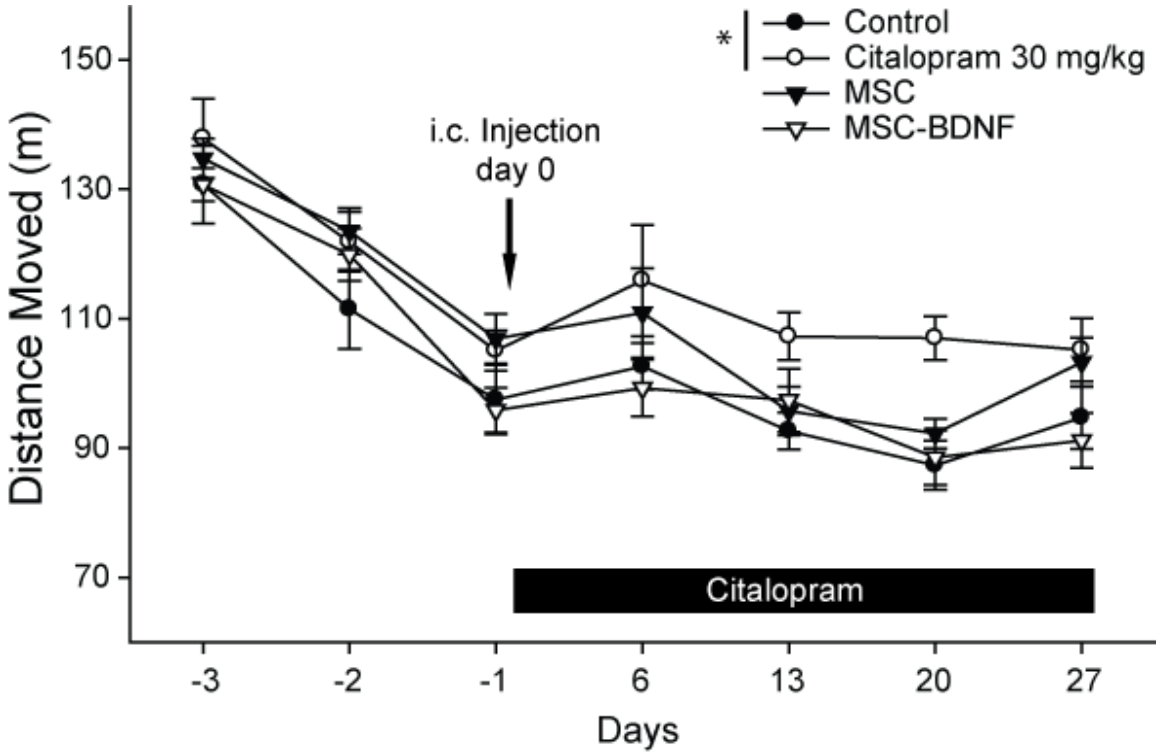

Figure 17: Depression-like behaviour in the Open Space Swim Test. A depression-like behaviour was induced during 3 days of 15 min swim sessions (days: -3, -2, -1). At day 0, MSC and MSC-BDNF were transplanted bilaterally into the hippocampus (i.c. injection) or citalopram treatment was initiated. Swim distance was recorded every 7 days for 4 weeks (days: 6, 13, 20, 27). Control n=11, citalopram n=13, MSC n=22, MSC-BDNF n=14. Results are expressed as means ± SEM. *P < 0.05 vs control, RM-ANOVA.

Stereotaxic transplantation of MSC (MSC and MSC-BDNF groups) or NaCl (control and citalopram groups) was performed the day after the third swim session, and citalopram treatment was initiated. The therapeutic effects against the induced depression-like behaviour was then determined through successive trials every 7 days for 4 weeks (Fig. 17). Analysis with RM-ANOVA of the four last consecutive swim sessions (days 6, 13, 20, 27) revealed no effects of MSC or MSC-BDNF transplantation on the induced depression-like behaviour, whereas citalopram-treated animals showed statistically improved swim distances ($P<0.05$).

3.3. MSC and MSC-BDNF have no long-term antidepressant effects in the LH model

Potential long-term protective effects of MSC and MSC-BDNF intrahippocampal transplantations against depressive-like behaviour were determined in the LH model of Depression. Animals underwent inescapable shocks on day 46 after transplantation, and were tested one day later in an active avoidance task. Analysis of escape latency to terminate the shock was performed and did not reveal any effects of MSC and MSC-BDNF transplantation against LH behaviour (Fig. 18A). In comparison, we found a statistically significant difference in LH behaviour between the citalopram-treated group and the control group ($P<0.05$). As internal control, animal activity was determined as the number of lever presses during the 15 trials and found to be was comparable between groups (Fig. 18B).

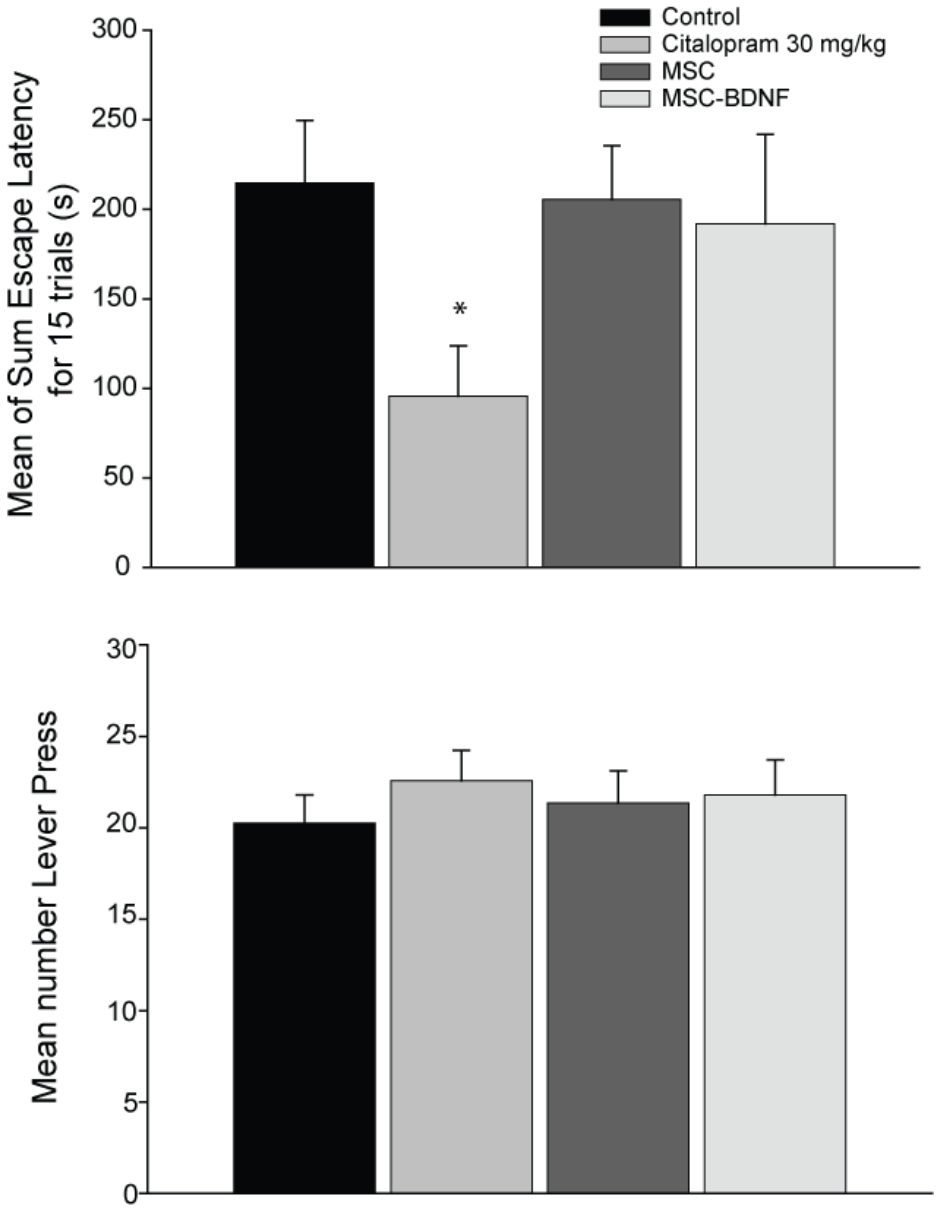

Figure 18: Depression-like behaviour in the Learned Helplessness paradigm. **A**: mean of sum latency to terminate the shock experience per animal during 15 trials. **B**: Number of lever presses during the active avoidance task was monitored as internal control for activity. Results are expressed as means ± SEM. Control n=12, citalopram n=12, MSC n=26, MSC-BDNF n=14. *P < 0.05 vs control, Mann-Whitney U test.

3.4. MSC and MSC-BDNF do not modulate general behaviours

To determine whether the transplantation might have induced other behavioural variations, locomotion in the OF scenario and anxiety-like behaviour in the EM were analyzed before OSST as baseline level, after OSST and after LH. For all time points analyzed, transplantation of MSC and MSC-BDNF showed no locomotion difference in comparison to control animals (Fig. 19A). Interestingly, 1W-ANOVA analysis revealed a significant increase of locomotor activity for the citalopram-treated animals after OSST (citalopram: 38.72 ± 2.76 m, control: 28.06 ± 2.72 m, $P<0.05$). After LH, the tendency toward increased locomotion was maintained in the citalopram group, although it did not reach statistical significance any more (citalopram: 37.02 ± 2.26 m, control: 29.53 ± 2.13 m, P=0,064). As internal control, baseline locomotion measurement before OSST was comparable between citalopram-treated animals and control animals (citalopram: 28.21 ± 3.26 m, control: 24.70 ± 2.69 m) and confirmed the specificity of the increase in locomotion by citalopram treatment.

Anxiety-like behaviour in the EM paradigm was then determined. Analysis of the percentage of open arm entries revealed no differences between the four groups (Fig. 19B).

As the transplantation targeted the hippocampal formation, effects of MSC and MSC-BDNF transplantation on hippocampal-dependent memory acquisition were assessed in the RAM paradigm. At 35 days after transplantation, a modified version of the protocol developed by Jarrard (1983) was used to assess working and reference memory acquisitions, two forms of hippocampal-dependent memory. RM-ANOVA revealed comparable memory acquisition through successive testing days for reference memory determined by the number of reference memory errors (Fig. 20A), as well as for working memory determined by the number of working memory errors (Fig. 20B) (for each group, $P<0.05$ within trials). Dunett post hoc test failed to reveal any differences between MSC, MSC-BDNF and citalopram groups in comparison to control animals. Thus, transplantation of MSC and MSC-BDNF did not impair hippocampal-dependent function in learning and memory.

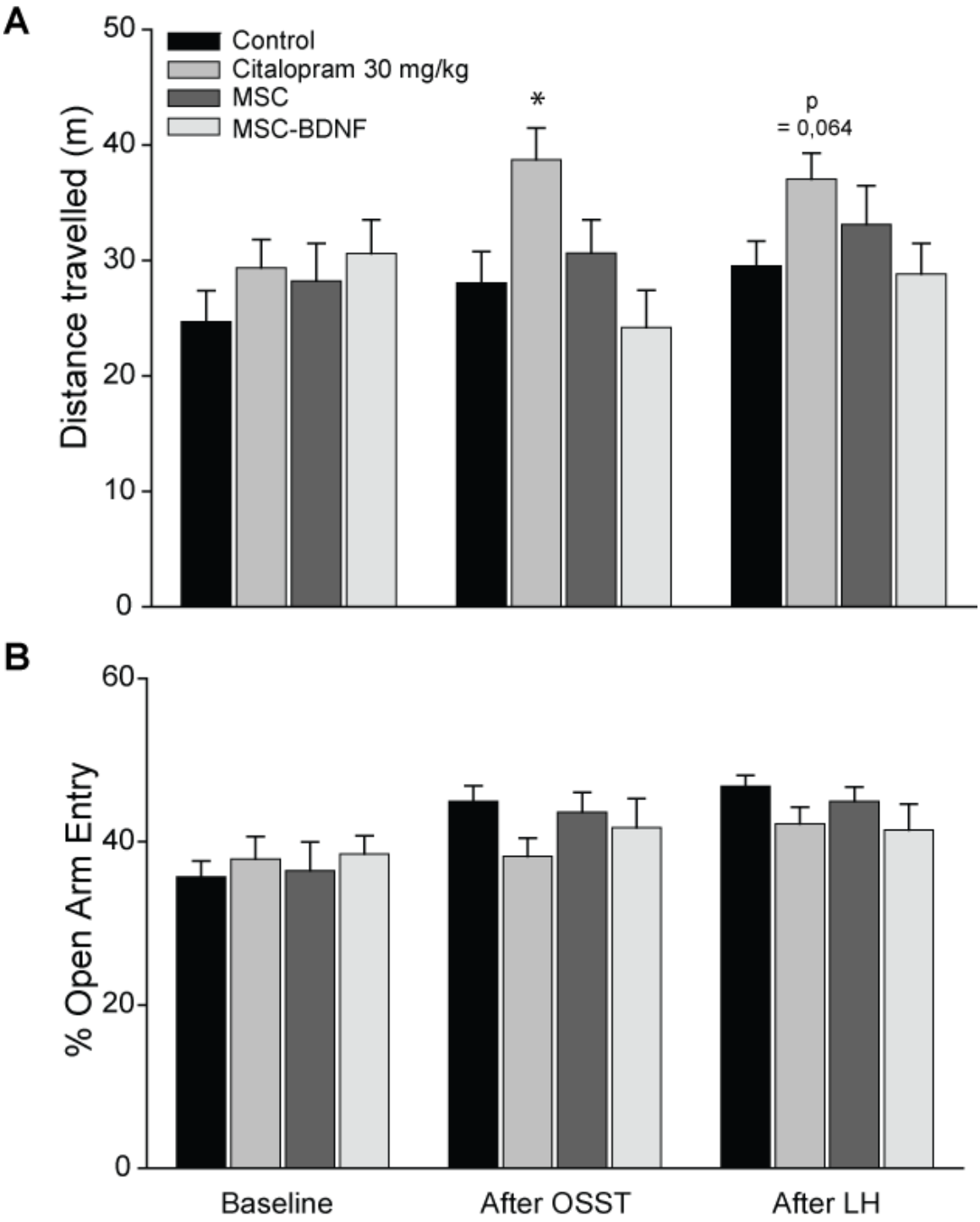

Figure 19: Analysis of general behaviours. **A**: Locomotion in the Open Field (OF) and **B**: anxiety-like behaviour in the elevated plus maze (EM) were performed before OSST, after OSST and after LH. Locomotion was determined as the total travelled distance in meter and anxiety-like behaviour was determined as the percentage of total number of entries in the open arm. Control n=12, citalopram n=14, MSC n=16, MSC-BDNF n=14. Results are expressed as means ± SEM. *p < 0.05 vs control, 1-way ANOVA.

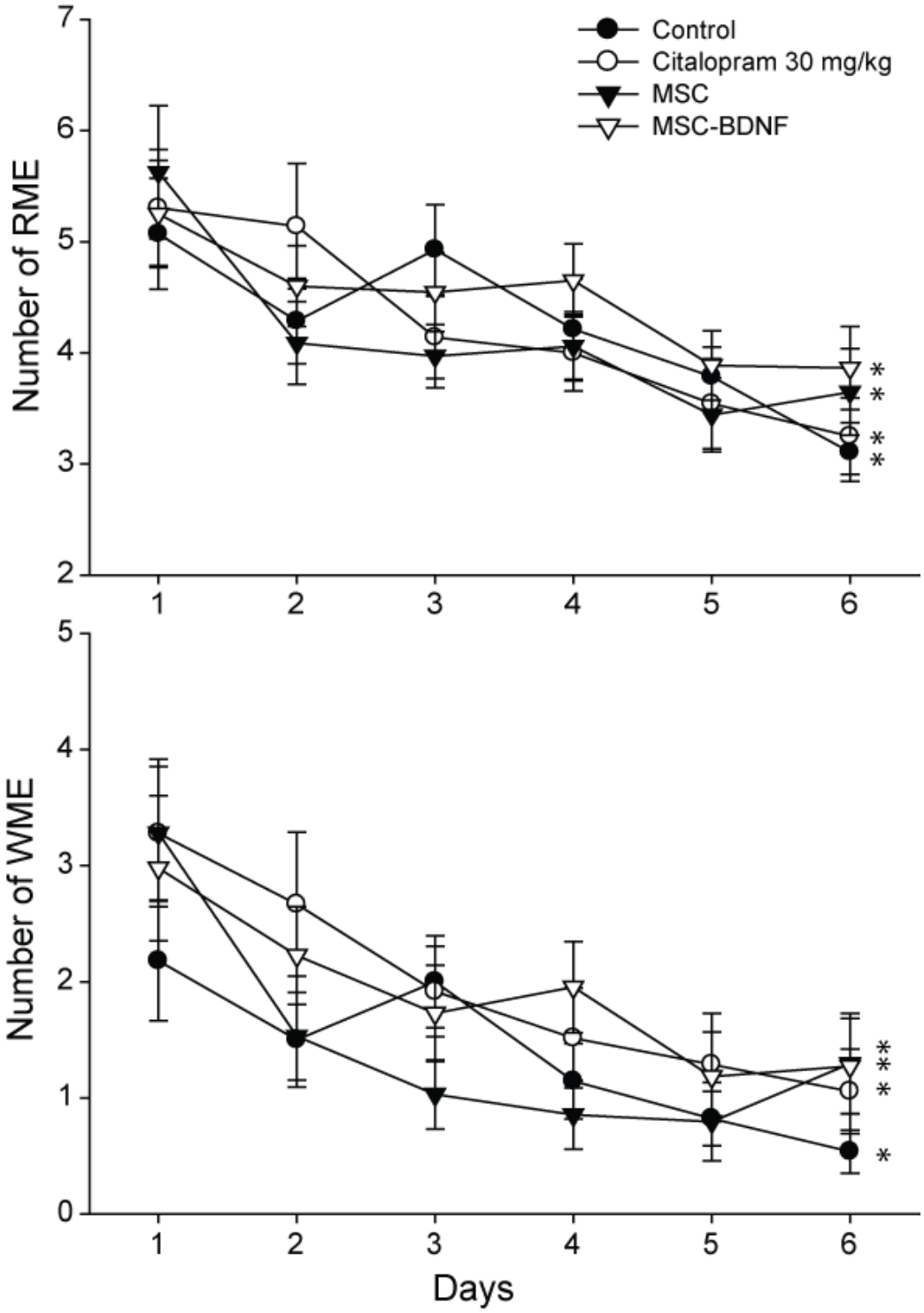

Figure 20: Memory performance in the 8-arm Radial Maze. **A**: Number of reference memory errors (RME) per test day and **B**: number of working memory errors (WME) per test day. Control n=14, citalopram n=18, MSC n=21, MSC-BDNF n=14. Results are expressed as means ± SEM. *p < 0.05 for RM-ANOVA, significant learning performance in each group between days 1-6, no differences between the groups.

3.5. MSC increase hippocampal cell proliferation and survival

Although the behavioural analysis failed to detect antidepressant effects, the consequences of MSC and MSC-BDNF transplantation on hippocampal structural plasticity were assessed. Two thymidine analogues were administered: BrdU and IdU, which can be differentially detected by immunohistochemistry. The level of cell proliferation and cell survival in subgranular zone (SGZ) and in the granular cell layer (GCL) of the dentate gyrus (DG) were thus determined. As expected, BrdU-positive clusters, representing proliferative cells, were mainly distributed in the SGL, whereas IdU-positive cells, representing surviving cells aged 31 days, were distributed both in the SGL and GCL (Fig. 21A, B and C).

Statistical analysis of BrdU-positive proliferative clusters and IdU-positive surviving cells per slice revealed statistically significant increases in the MSC and MSC-BDNF transplanted groups compared to control (**IdU**; control: 4.36 ± 0.18, MSC: 4.87 ± 0.19, P=0.024; MSC-BDNF: 4.85 ± 0.18, P=0.008 ; **BrdU**; control: 2.92 ± 0.11, MSC: 3.77 ± 0.14, P<0.001; MSC-BDNF: 3.52 ± 0.12, P<0.001) (Fig. 21D). For the citalopram treatment group, increased cell survival was observed (4.74 ± 0.19, P=0.03) (Fig. 21D), whereas no difference was found for BrdU-positive proliferative clusters compared to control (3.07 ± 0.12). Thus, transplantation of MSC, and not the additional ectopic expression of BDNF, induced a long-term stimulation of hippocampal cell proliferation, which probably gave rise to the apparent increase of cell survival. In contrast, citalopram increased only the rate of cell survival.

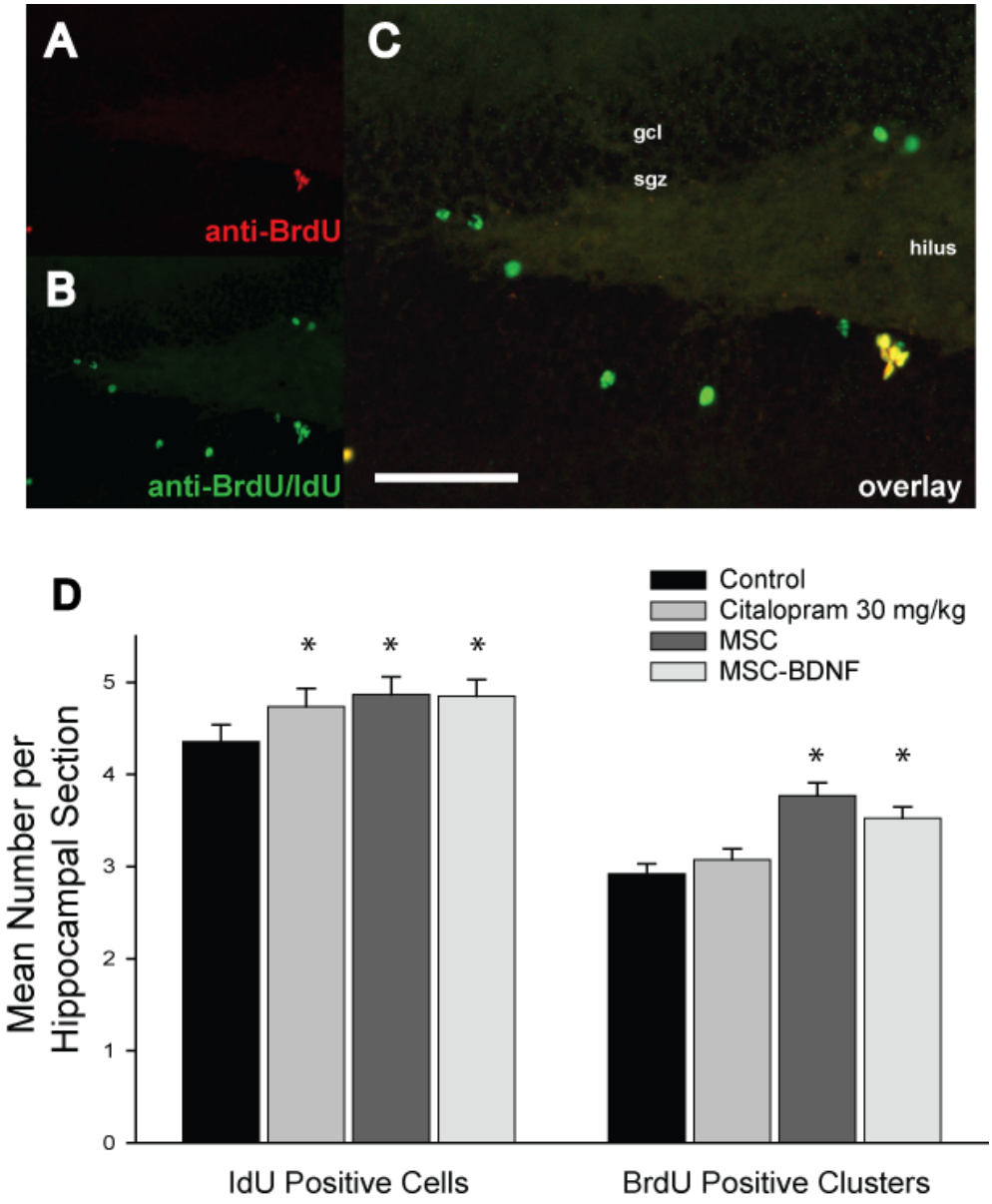

Figure 21: Hippocampal cell survival and proliferation. Proliferative BrdU-positive cells (**A**: red) and surviving cells (**B**: green) in the DG. **C**: Merged picture allows the detection of proliferative surviving cells (yellow, BrdU/IdU double positive) and surviving, non-proliferative IdU-positive cells in the same slice (green). **D**: Quantification of BrdU-positive clusters and IdU-positive cells per slice for each group. Total analyzed slices per group: Control n= 336 for 10 animals, citalopram n= 255 for 8 animals, MSC n= 312 for 9 animals, MSC-BDNF n= 313 for 9 animals. SGZ: Sub-Granular Zone, GCL: Granular Cell Layer. Scale bar = 200 µm. *p < 0.05 compared to control using the Mann-Whitney U test.

3.6. Rapid loss of MSC graft accompanied by activation of innate immunity

Due to the lack of effect on depression-like behaviour in the OSST and LH, and on hippocampal-dependant function in the RAM, we asked whether the transplanted MSC were still present during the different behavioural tasks and whether BDNF expression was still effective. The engraftment properties of VSOP-labelled MSC-GFP and the corresponding MRI signals were determined. 30 000 VSOP-labelled MSC-GFP were transplanted into each hippocampus, and MSC distribution and microglia activation were analyzed by immunohistochemistry at 1, 6, 20 and 50 days after transplantation (n=2 for each time point).

VSOP-labelled MSC-GFP were well integrated into the host tissue at 1 day after transplantation and showed a normal MSC morphology (Fig. 22A). MSC-GFP were distributed in clusters in all hippocampal subregions similar to the figure 13D (data not shown). At that time point, only a minor inflammatory response in the core of the graft was observed as determined by the presence of Iba1-positive cells (Fig. 22A). Not all cells present in the graft were expressing GFP as indicated by comparison with the distribution of DAPI-positive nuclei. In contrast, at 6 days after transplantation, MSC-GFP grafts were strongly infiltrated with Iba1-positive cells (Fig. 22B). Although MSC morphology was maintained, fewer MSC-GFP were observed in comparison to day 1, suggesting host rejection of engrafted MSC. At 20 days after transplantation, some occasional MSC-GFP were detected, but none were found after 50 days, suggesting a total clearance of GFP-positive MSC. Numerous DAPI-stained nuclei were nevertheless detected in the remaining core of the graft at 50 days after transplantation (data not shown). Finally, MRI acquisitions were similar as these depicts in figure 13A-C.

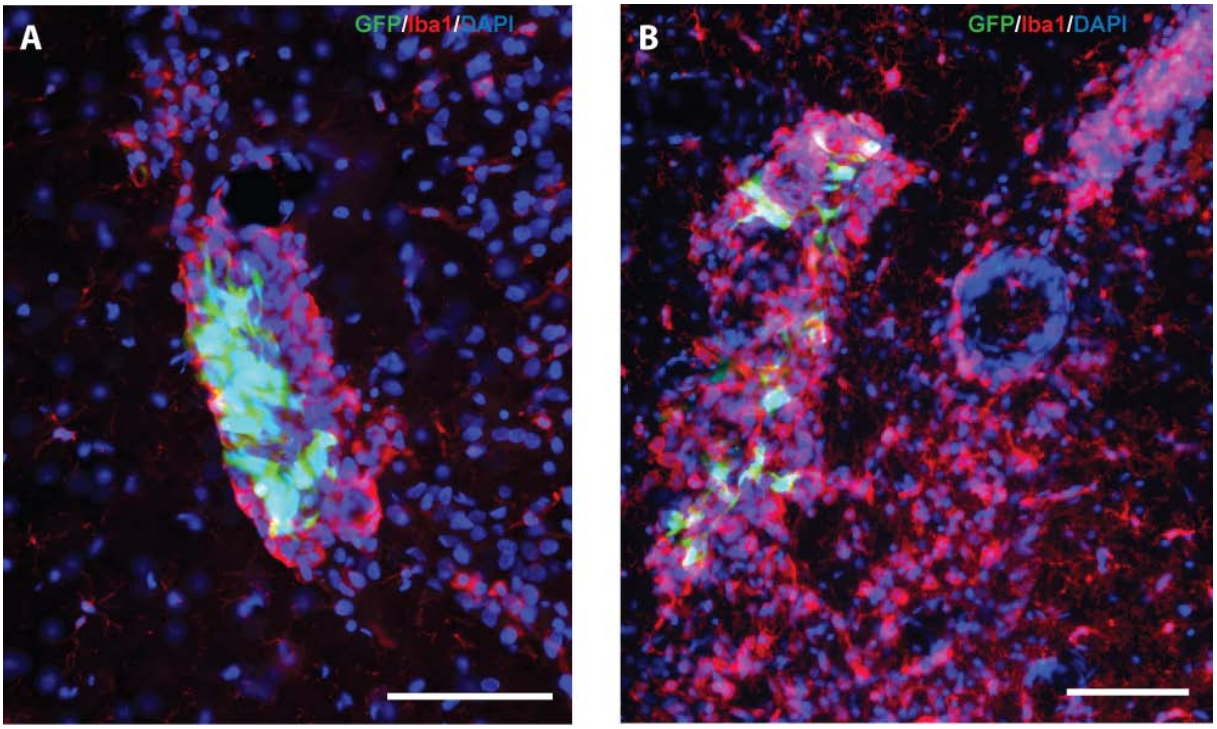

Figure 22: MSC engraftment and rejection. **A**: GFP-expressing MSC graft (green) at 1 day after transplantation surrounded by discrete infiltration of Iba1-positive microglia (red). Nuclei are stained with DAPI (blue). **B**: Massive recruitment of Iba1-positive microglia at 6 days after transplantation with few remaining GFP-positive MSC, but persistence of DAPI positive nuclei in the core of the graft. Scale bars = 100 µm.

IV. Discussion

In this study, several methods were used to assess the effects of intrahippocampal transplantation of MSC and BDNF-expressing MSC on depressive-like behaviour in the rat. Allogeneic transplantation of Lewis MSC into Lewis rats was performed without immunosuppression. MSC were first labelled with VSOP particles in order to determine *in vivo* the distribution of MSC after intrahippocampal transplantation. Efficiency of VSOP-labelling and its impact on MSC properties were assessed *in vitro* and *in vivo*. For the behavioural study, MSC were genetically modified with a lentivirus in order to express BDNF and/or GFP. After intrahippocampal transplantation, depression-like behaviour was assessed using two validated models of Depression, and the specificity of any behavioural changes were controlled by additional behavioural tasks. Hippocampal structural plasticity was also studied given its implication in depression-like behaviour. Finally, MSC engraftment kinetics were analyzed and rapid graft rejection was observed.

1. *In vitro* MSC labelling with VSOP

Our results demonstrate that Lewis rat MSC can be successfully labelled *in vitro* with the superparamagnetic particles VSOP. In agreement with the studies of Stroh et al. (2004; 2005), VSOP labelling did not affect cell viability or MSC proliferation and differentiation potential. Efficient MSC labelling has previously been described with several classes of contrast agents (Ittrich et al. 2005), however, in comparison with other cell types such as embryonic cortical neurons, macrophages or a microglial cell line, VSOP incorporation into MSC seemed to be limited and did not reach the maximal achievable reduction of T2* relaxation time (personnal communication by A. Stroh and experimental observations from our laboratory). The limited particles loading capacity of MSC might further impair MRI detection, when *in situ* cell division occurs. As an alternative approach, transfection agents (Souery et al. 2006) might be added to increase contrast agent load during labelling (Arbab et al. 2004). Although transfection agents can disturb the integrity of the cellular membrane, this approach is currently widely used for cells with limited contrast agent loading and endocytosis rates such as the MSC.

Incorporation of VSOP was nevertheless sufficient to detect MSC with MRI in an agarose gel phantom, although the detection of the hypointense signal was limited to 100 labelled cells and the spatial resolution was also limited with the 4,7 T magnetic field we used. It therefore remains to be determined whether, with our labelling characteristics, single cell detection using higher magnetic fields might be feasible, as described for 17,5 T (Stroh et al. 2005).

2. MSC lentiviral transduction and BDNF expression

Our main goal was to determine the effects of intrahippocampal transplantation of MSC and BDNF-expressing MSC on depressive-like behaviour. In order to achieve expression of BDNF, MSC were transduced with a lentiviral vector generously provided by Armin Blesch, which has already shown efficient functional recovery properties after local MSC-BDNF transplantation in a rat model of spinal cord injury (Kwon et al. 2007). In this virus, the GFP sequence was inserted after an IRES, which allows for histological cell detection of BDNF-expressing cells. As a valuable control for the behavioural study, MSC were also transduced with the same lentiviral vector encoding only for GFP. After transduction, the GFP-expressing MSC population was selected by fluorescent-activated cell sorting. Persistence of GFP expression was confirmed up to 2 passages after cell sorting. The level of BDNF expression in MSC was comparable with the one determined by Armin Blesh (personnal communication) and with a previous study using an adenoviral vector in MSC (Kurozumi et al. 2004; 2005).

3. *In vivo* MRI cell tracking of VSOP-labelled MSC

As an innovative strategy, we used *in vivo* imaging with high resolution MRI to determine MSC migration patterns and MSC graft volumetric distribution after intra-hippocampal transplantation. VSOP-labelled MSC were transplanted and hypointense signal distribution was analyzed in a time course manner. Although a reduction in signal strength was observed 20 and 50 days after transplantation, graft localization remained constant and matched Prussian Blue histology for detection of iron. Interestingly, no significant migratory pattern was detected even 6 days after transplantation. Using similar *in vivo* imaging modalities, numerous studies have shown migratory paths for MSC in lesioned tissue environment (Jendelova et al. 2003, 2004; Sykova and

Jendelova 2007; Wu et al. 2008). In our study, maintenance of the brain parenchyma integrity may have supported stable graft localization, presumably due to the absence of migratory cues (Guzman et al. 2007). Application of MRI for high resolution cell tracking is thus a suitable method to determine the spatial engraftment characteristics in cell replacement strategies or for targeted cell-mediated gene therapy. Here, an isotropic FLASH sequence was used with long acquisition duration of about 1 hour. For high throughput screening, this method seems to be limited and further optimization to shorten the scan acquisition duration might facilitate the use of MRI cell tracking for routine applications.

4. Depression models and other behavioural tasks

We used two validated models of Depression and additional behavioural tests to assess depression-like behaviour in rats. The main goal of this study was to assess the effects of intrahippocampal transplantation of MSC and BDNF-expressing MSC on depressive-like behaviour. For this reason, the OSST model was chosen due to its advantage of being able to induce and maintain a depression-like behaviour and subsequently assess recovery for 4 weeks after induction. This model has already been used successfully for this purpose in rats (Sun and Alkon 2004) and more recently in mice (Stone et al. 2008). In those studies, induced depressive-like behaviour was reversed with AD administration, including SSRI, in a time manner corresponding well with the delay of AD efficacy observed in human patients. We reproduced these observations by treating rats with the SSRI citalopram added to the drinking water at a concentration of 30 mg/kg, which has previously been shown to be efficacious in rats (Rygula et al. 2006). Despite of its advantage with regard to the face validity, the OSST, as a derivative of the FST, only reproduces some aspects of the human Depression phenotype (Cryan et al. 2005). Thus, negative findings in the OSST should not lead to the rejection of a new antidepressant therapy. For example, trials in mice with SSRI have initially failed to detect any antidepressant effects with the conventional Porsolt FST paradigm (Cryan et al. 2005). Given that SSRI are currently the most prominent ADs with low concomitant side effects, only extensive research to modify the FST led to the efficient detection of the antidepressant capacity of these drugs. A modern strategy en route to new therapeutics against Depression therefore must rely on the parallel or consecutive use of several animal models of Depression. For this reason, the Learned Helplessness

model of Depression was also used in our study as developed by Vollmayr (Vollmayr and Henn 2001). This model is widely accepted as the most reliable rat model of human Depression (Henkel et al. 2002). However, similar to the OSST, interpretations derived from this model may be affected by locomotion deficits, cognitive performance and anxiety-like behaviour. For example, motor hyperactivity may have an influence on the frequency of lever presses in the LH paradigm, resulting in less helpless behaviour as expressed by an increase of lever presses.

We therefore added a battery of behavioural tests to the models of Depression. Locomotion was assessed in the open field, anxiety in the elevated plus maze and memory acquisition in the 8-arm Radial Maze. The OF and EM were performed initially prior to the OSST to rule out that particular behavioural traits were selected in each experimental group. Tests were also performed after the OSST and LH models to determine whether the swim distance and the latency to press the lever, respectively, were specific to depression-like behaviour. In contrast to the OF and EM, the RAM procedure can not be applied several times. For this reason, we performed a single series after the OSST model. In addition to the locomotion determination with OF, activity in the Skinner box, during the active avoidance task of the LH model, was determined by counting the number of lever presses and thus refined the impact of locomotion in this task. Despite the extensive efforts needed for the screening of additional behaviour traits, such an approach is highly recommendable to assess a specific behaviour related to mood disorders by revealing any experimental or interpretative confounding factors.

5. Absence of behavioural effects of MSC transplantation

We found that neither MSC transplantation, nor transplantation of BDNF-expressing MSC resulted in any antidepressant therapeutic effects in the OSST model or preventive effects in the LH model. Locomotion, anxiety-like behaviour and hippocampal-dependent learning/memory were also unchanged compared with the control group. By analyzing brain sections after completion of the behavioural study, no GFP-positive MSC were detected at the expected site of engraftment as determined by MRI cell tracking. The engraftment kinetics of GFP-expressing MSC were subsequently assessed, and innate immune responses were monitored by immunostaining of microglia/macrophages. A strong infiltration of Iba-1 positive cells was detected in the

MSC-GFP graft with a peak at 6 days after transplantation, resulting in complete graft rejection at 50 days after transplantation. The loss of MSC might in turn explain the slight diffusion of the MRI signal, which has already been observed after transplantation of non-viable, SPIO-labelled embryonic stem cells (Guzman et al. 2007). In contrast to the loss of the GFP signal, Prussian Blue histology suggested full integration and maintenance of the MSC graft even after 50 days, suggesting that functional markers, such as GFP or LacZ reporter genes, are required to complement the histological validation of *in vivo* MRI cell tracking.

Interestingly, previous studies on MSC transplantation in the Central Nervous System (CNS) have yielded contradictory results with regard to MSC engraftment, integration and subsequent beneficial effects in pathological environments (Chen et al. 2001; Bae et al. 2007; Wu et al. 2007). All of these studies differ significantly from our experimental paradigm. First, MSC transplantation was performed mainly into lesioned or degenerated CNS, with or without immunosupression using cyclosporine. Here, an allogeneic transplantation strategy was performed in non-immunosuppressed rats, and MSC were transplanted into healthy CNS. Thus, the engrafted cells may have been more susceptible for local immune rejection. Additionally, a significantly lower number of cells was transplanted in our study compared to previous studies, which may have increased the accessibility of the engrafted cells for local immune responses. Finally, MSC are well tolerated and show a widespread distribution after transplantation into neonatal brain, but this tolerance seems to decrease with age (Phinney et al. 2006).

Interestingly, two previous studies on allogeneic MSC transplantation into normal adult rat brain (Coyne et al. 2006; 2007) showed MSC graft rejection comparable with our study. In contrast, Phinney et al. (2006) observed long-term male MSC engraftment in adult female mice, although with a lower rate of integration in comparison to neonatal mice. In this study, determination of cell integration was performed using quantification of male DNA or Y chromosome immunodetection, but no functional evidence for MSC persistence was provided. Further studies are required to assess the feasibility of autologous MSC transplantation in normal adult brain, in particular in light of their use as cellular vectors for gene therapy. As an alternative strategy to the direct transplantation of MSC, encapsulation with biocompatible materials, such as alginate microcapsules, might be a promising approach to protect MSC from local immune responses and to allow long-term ectopic gene expression (Orive et al. 2003, 2006; Herrero et al. 2007).

6. Absence of behavioural effects of BDNF overexpression

Previous studies have demonstrated that intrahippocampal BDNF injections provide robust antidepressant effects in the FST and LH models of Depression. In those studies, maintenance of the antidepressant effects was only assessed for up to 10 days in the LH paradigm with 3 repeated injections (Shirayama et al. 2002), or for up to 6 days after a single injection in the modified FST paradigm (Hoshaw et al. 2005). In our study, transient ectopic expression of BDNF prior to graft rejection might have been sufficient to drive long-term antidepressant effects. However, we failed to detect any such effects. In addition to the apparent loss of MSC within on week, several other differences in our experimental paradigm may explain the discrepancies. First, MSC-BDNF may not have secreted enough BDNF to induce antidepressant effects. In fact, BDNF-transduced MSC secreted less than 1 ng per 30 000 cells within 24h *in vitro*. In comparison, the minimal effective antidepressant dose of injected BDNF used in the study by Shiriyama et al. (2002) was about 250 ng. We had decided to transplant low MSC cell numbers in order to keep the structure of the hippocampal formation largely intact, but in future, higher numbers of MSC may have to be transplanted or the viral vector may have to be further optimized for higher transgene expression. Second, we specifically targeted the middle of the hippocampus in contrast to previous studies. However, the ventral hippocampus is currently thought to be mainly involved with emotion processing (Kjelstrup et al. 2002; Bannerman et al. 2004). Thus, further studies are needed to clarify whether the antidepressant actions of local BDNF administration are confined to certain areas of the hippocampus.

7. Modulation of hippocampal structural plasticity by MSC

It has been recently proposed that MSC are able to stimulate their surrounding environment after transplantation (Crigler et al. 2006; Phinney and Prockop 2007). Indeed, we found that MSC transplantation modulated long-term hippocampal structural plasticity. Hippocampal cell proliferation and cell survival were increased; the latter observation might be hypothesized to result from the global increase of proliferation. In contrast to our findings, Munoz et al. (2005) suggested that MSC promote hippocampal proliferation for only up to 7 days after transplantation. However, Munoz et al. (2005) transplanted human MSC into mice, and assessed cell proliferation in the hippocampus

by mRNA quantification of the nuclear proliferative marker, PCNA. Also, we performed behavioural tasks with the rats, which are known to stimulate hippocampal plasticity, and this may have provided the grounds for the longer term effects of MSC.

Interestingly, despite the observed modulation of cell proliferation in the DG, no amelioration of depression-like behaviour by MSC was observed in the OSST and LH models. As originally described by Malberg et al. (2000), chronic AD treatment increases cell proliferation and survival. Here, long-term treatment with citalopram increased hippocampal cell survival without altering the cell proliferation rate. This discrepancy may result from our experimental paradigm, in which a battery of behavioural tests was performed that might have modulated to some degree animal cognition and hippocampal physiology. Chronic swim sessions in the OSST can be considered to represent a repeated physical exercise, which is known to increase hippocampal cell proliferation (van Praag et al. 1999). In addition, a learning task was performed with the RAM procedure, and learning is known to increase cell proliferation in the hippocampus (Gould et al. 1999). This could have concealed some of the citalopram effects on hippocampal neuroplasticty. Nevertheless, it is tempting to speculate from our data and previously published results (Santarelli et al., 2003) that SSRIs exert antidepressant effects via improved survival of newly generated cells in the dentate gyrus in combination with yet unidentified additional actions, which MSC are unable to provide in our study.

8. Conclusion

Our study provides evidence that MSC can be labelled with VSOP and tracked *in vivo* using MRI. However, we strongly emphasize the use of functional markers, such as GFP, to validate the MRI signal. Bilateral intrahippocampal transplantation of 30,000 MSC resulted in transient engraftment, followed by rapid immune rejection of MSC in the healthy adult brain. Intrahippocampal transplantation of MSC promoted long-term changes in hippocampal structural plasticity, but failed to provide antidepressant effects in two validated rat models of depression-like behaviour.

V. Summary

Early hypotheses on the pathophysiology of major Depression were based on aberrant intrasynaptic concentrations of the neurotransmitters Serotonin and Norepinephrine. However, a number of recent studies suggested that changes in hippocampal neuroplasticity may underlie the development of Depression and the recovery from disease. Moreover, brain-derived neurotrophic factor (BDNF) has been proposed as one of the most promising molecular targets to stimulate hippocampal neuroplasticity and to reverse Depression phenotypes.

We hypothesized that intrahippocampal transplantation of mesenchymal stromal cells (MSC) from bone marrow, which are genetically engineered to express BDNF, might offer a promising approach for the treatment of depression-like behaviour in rodents by increasing hippocampal neuroplasticity. We used two validated models of Depression: the Open Space Swim Test and the Learned Helplessness paradigm. In addition, a battery of behavioural tests was performed, namely screening of learning and memory with the 8-arm Radial Maze, locomotion with the Open Field test and anxiety with the Elevated plus Maze. MSC were labelled with a magnetic contrast agent (VSOP) and the transplanted cells were tracked *in vivo* using Magnetic Resonance Imaging (MRI). With the help of the thymidine analogues, BrdU and IdU, effects of MSC transplantation on hippocampal structural plasticity were assessed by determination of cell proliferation and survival in the Dentate Gyrus of the hippocampus.

Intrahippocampal transplantation of MSC promoted long-term changes in hippocampal structural plasticity, but failed to provide antidepressant effects in both models of depression-like behaviour. The most likely explanation for our findings is the rapid immune rejection of the transplanted allogeneic MSC in the adult brain.

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Appendix

1. Equipment

Table2: Equipment

Application	Name	Supplier	Description
Cell Culture	Bench KR-130 BW	Kojair	
	Incubator MCO_18AIC	Sanyo	
	Water bath SW22	Julabo	
	Centrifuge Universal 320R	Hettich Zentrifugen	
	Pump Vaccubrand	Wertheim Germany	
	Microscope CKX41	Olympus	
FACS	Calibur®	BD Biosciences	FACS
	Diva®	BD Biosciences	Cell sorting
Animal experiments	Conventional Animal Facility		
	3 Behavioural boxes 2 m2		
	Stereotaxic Frame	TSE Biosystem	
	Warming plate		After OP
	Rat Guillotine		Sacrifice
	Perfusion Sytem		
	Open field plate 1x1m	Home made	Locomotion
	Elevated Plus maze	TSE systems	Anxiety
	8-arm Radial Maze	TSE systems	Learning Memory
	Skinner Box for Learned Helplessness	TSE systems	LH depression paradigm
	Swimming Pool, 156 cm diameter	TSE systems	OSST depression paradigm
	Video Acquisition Card TSE videomot, connected to personal computer	TSE systems	Animal tracking
	2 cameras V3 1316	TSE systems	Animal tracking
	Video Recorder VR 630	Philips	Animal tracking
	Water Pump	Jung Pumpen	OSST depression paradigm

Application	Name	Supplier	Description
Molecular Biology	Agitator SM30	Johann Otto GmbH	
	Incubator HT	Infors	
	Ice AF80	Scotsman	
Brain Sectioning	Vibratome HM650 with cooling system CU95	Microm	Graft analysis
	Microtome SM2000R	Leica	Brain sectionning
Histochemistry	Bench Cruma870	Cruma	
Microscopy	Microscope DMRA2 Control Unit CTR MIC Fluorescence Unit ebq100	Leica	
	Leica with stereology	Leica	Stereology
Magnetic Resonance	Pharmascan 7 T	Brucker Biospin	
	NMR Spectrometer	Brucker Biospin	
Software	Videomot®	TSE systems	Video Tracking
	ImageJ		Image analysis
	Amira®	T65 – San Diego	Image analysis
	Matlab®	MathWorks	Image analysis
	SPSS	SPSS	Statistics
	Sigmastat	Sigma	Statistics

2. Reagents

Table 3: Reagents

Application	Solution	Name	Working concentration and solvent	Supplier	Catalog. Number
Cell Culture Media and Buffer	10% or 20%-MSC Medium (depending on FCS)	Alpha MEM		Biochrom AG	F0915
		Fetal Cow Serum "serum supreme"	10% or 20%	BioWhittaker ; Cambrex	14-429E
		L-Glutamine 200 mM	2 mM	Biochrom AG	K0283
		Penicillin Streptomycin	100 U/mL 100 µg/mL	Biochrom AG	A2213
		Trypsin/EDTA 10X	1X	Biochrom AG	L2153
		Phophate-Buffer Saline (PBS) 10 X -MgCl2 -CaCl2	1X	Gibco	14200
		Sterile Water		Fresenius Kabi	Ampuwa
	Frozen Medium	Alpha MEM		Biochrom AG	F0915
		Fetal Cow Serum "serum supreme"	30%	BioWhittaker Cambrex	14-429E
		DMSO	5%	Biochrom AG	
	Fixation	Formalin 10 % in solution	10 %	Sigma	HT50-1-128
	Counting Viability	Trypan Blue 0,5% w/v	0,05% w/v, PBS	Biochrom AG	L6323
Magnetic Labelling		VSOP-C200 500 mM	0, 1,5, 3, 6, 9 mM	Ferropharm, Teltow	
MRI gel phantom		Agarose NEEO ultra qualitaet	1,5 %, TBE	Roth	2267

Application	Solution	Name	Working concentration and solvent	Supplier	Catalogue Number
Differentiation Media		10%-MSC Medium			
	Adipogenesis	100 µg/mL 3-isobutyl-1-methylxanthine (IBMX)	100 µg/mL, Methanol	Sigma	17378
		insulin	5 µg/mL,water pH 2 with HCl 2 M	Sigma	16634
		Dexamethasone	1 µM, H20	Sigma	D2915
		Indomethacine	60 µM, H2O	Sigma	17378
	Osteogenesis	Glycerophosphate	10 mM, H20	Sigma	G9891
		Dexamethasone	10 nM, H20	Sigma	D2915
		Ascorbate-2-phosphate	200 µM, H20	Sigma	A8960
Differentiation Staining	Osteogenesis	Alizarin Red S	1 %, water, pH 4,1 with 0,1 % Ammonium Hydroxid	Sigma	A5533
	Adipogenesis	Oil Red O	0,5%, Methanol	Sigma	198196
Lentiviral Transduction		LV-GFP $5.5\ 10^6$ particles/mL	20 and 50 m.o.i		
		LV- BDNF-GFP $3.5\ 10^6$ particles/mL	20 and 50 m.o.i		
		Polybrene	6 µg/mL		
Antidepressant Drug		Citalopram 30 mg/mL (Cipralex®)	30 mg/kg, tape water	Lundbeck GmbH	
Blood sample		Heparin 5000 U/mL	25 µL/sampled tubes	Biochrom AG	
Thymidine Analogues		IdU	50 mg/kg	Sigma	I7125
		BrdU	50 mg/kg	Sigma	B5002

Application	Solution	Name	Working concentration and solvent	Supplier	Catalog Number
Anaesthesia	Stereotaxic Operation	Pentobarbital	50 mg/kg, NaCl 0,9%	Sigma	P3761
	MRI scan	Isoflurane	1,5 to 2,5 %, O2		
	Sacrifice	Chloral Hydrate	400 mg/kg, NaCl 0,9%	Merck	1.02425
Post-operative analgesia		Novamine-Sulphon (Metamizol)	50-100 µL/rat/day	Winthrop	
Perfusion		Paraformaldehyde	0, 4%, PBS pH 7,4	Sigma	P6148
Brain processing		Dulbecco's Phosphate Buffer Saline (PBS)	0,1 M, pH 7,4 (HCl)	Sigma	D57773
		Sucrose	30%, H20	Sigma	S8501
	Conservation solution	Sodium Azide	0,5%, PBS	Roth	K305.1
	Cryoprotective Solution	Glycerol	50%	Sigma	G8773
		Ethylenglycol	25%	Sigma	
		PO4 0,1 M	25%	Sigma	
Histology	Prussian Blue Iron Staining	Ferrocyante 4 % w/v, water	1/3	Sigma	P9387
		HCl, 2 M	2/3	Sigma	

Application	Solution	Name	Working concentration and solvent	Supplier	Catalog Number
Immunohisto -chemistry	1st Antibodies	mouse monoclonal anti-BrdU	1/250	Becton Dickinson	34758
		rat monoclonal anti-BrdU	1/250	Serotec	MCA2060
		Iba-1	1/50	Wako	
	2nd Antibodies	anti-rat Texas Red	1:200	Molecular Probes	T6392
		anti-mouse Alexa fluor 488	1:200	Invitrogen	A21202
		anti-rabbit Texas Red	1:200	Molecular Probes	T6391
	Media	Serum	10 or 6 %		
		Triton X-100	0,1%	Sigma	T9284
		Borate Buffer	0,1 M pH 8,5 (HCL)	Sigma	18,509
		HCl	2 N		
		Vectashield DAPI mounting medium		Vector	H-1200
		Mowiol			
BDNF Expression		BDNF Emax® ImmunoAssay System		Promega	G7610

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Curriculum Vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht.

List of publications related to the thesis

Oersal AS., Blois S., Bermpohl D., Schaefer M., Coquery N. (2008) **Administration of interferon-alpha in mice provokes peripheral and central modulation of immune cells, accompanied by behavioral effects.** Neuropsychobiology, 58:211-222

Coquery N., Kwindzinski E., Bechmann I., Blesch A., Stroh A., Kempermann G., Winter C., Priller J. (2009) **Intrahippocampal transplantation of bone marrow-derived mesenchymal stromal cells promotes neuroplasticity without conferring antidepressant effects**. Submitted for publication, Exp Neuro

Winter C., Klein J., Coquery N., Mundt A., Morgenstern R., Kupsch A., Juckel G**. Nucleus subthalamicus is differentially involved in depression and anxious behavior as well as cognitive and locomotor performance in rats.** Manuscript in preparation

Meetings with poster /oral presentations related to the thesis

1. 05/2006 Polish-German Workshop for PhD Students, Warsaw, Poland
2. 06/2006 Spring School for Regenerative Medicine, Rostock, Germany
3. 11/2006 Berlin Brain Days, Berlin, Germany
4. 11/2007 Berlin Brain Days, Berlin, Germany

Others initiatives

Organization of the **Berlin Brain Days** students conference (2005, 2006, 2007): Communication, Internet Site (www.charite.de/medneuro/BBDpresentation)
Organization of the Biotechnology Forum (2003): Logistics, Communication

Nicolas Coquery

Eidesstattliche Erklärung

„Ich, Nicolas Coquery, erkläre, dass ich die vorgelegte Dissertationsschrift mit dem Thema: „Intrahippocampal transplantation of MSC and MSC-expressing BDNF in Rat Models of Depression-like Behaviour“ selbst verfasst und keine anderen als die angegebenen Quellen und Hilfsmittel benutzt, ohne die (unzulässige) Hilfe Dritter verfasst und auch in Teilen keine Kopien anderer Arbeiten dargestellt habe.“

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