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# DISSERTATION

# "Mesenchymal stem cell characterization and effects on motor and non-motor symptoms in a Parkinson's disease rat model"

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**Anne Schwerk**

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# Mesenchymal stem cell characterization and effects on motor and non-motor symptoms in a Parkinson's disease rat model

Anne Schwerk[1], Jennifer Altschüler[1], Manfred Roch[2], Manfred Gossen[2, 3], Christine Winter[4], Jürgen Berg[1], Andreas Kurtz[2, 3, 5] , Levent Akyüz[2, 6], Barbara Steiner[1*]

[1] Department of Neurology, Charité University Medicine; [2] Berlin-Brandenburg Center for Regenerative Therapies (BCRT), Berlin, Germany; [3] Helmholtz-Zentrum Geesthacht (HZG), Institute of Biomaterial Science, Teltow, Germany; [4] Department of Psychiatry, Technical University Dresden; [5] Seoul National University, College of Veterinary Medicine and Research Institute for Veterinary Science, Seoul 157-742, Republic of Korea, [6] Institute for Medical Immunology, Berlin, Germany.

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## 1. List of abbreviations

| | |
|---|---|
| + | positive |
| 6-OHDA | 6-hydroxydopamine |
| µg | microgram |
| µl | microliter |
| µm | micrometer |
| AB | antibody |
| AM | arachnoid mater |
| ANOVA | analysis of variance |
| AP | anterior–posterior |
| BDNF | brain-derived neurotrophic factor |
| BrdU | 5-bromo-2-deoxyuridine |
| BrdU+/HuD+ | BrdU and HuD double-positive cells |
| CD | cluster of differentiation |
| DAB | 3,3'-diaminobenzidine |
| DAPI | 4',6-diamidino-2-phenylindole |
| DNA | deoxyribonucleic acid |
| DV | dorsoventral |
| EPO | erythropoietin |
| GDNF | glial cell line-derived neurotrophic factor |
| GFAP | glial fibrillary acidic protein |
| GFP | green fluorescent protein |
| h | hour |
| HuMi | human mitochondria |
| iba1 | ionised calcium-binding adapter molecule 1 |
| IL | interleukin |
| i.p. | intraperitoneal |
| IPS | idiopathisches Parkinsonsyndrom |
| kg | kilogram |
| M | molar |
| MAP2b | microtubule associated protein |
| MFB | medial forebrain bundle |
| mg | milligram |
| MHC-I | major histocompatibility complex class I |

| | |
|---|---|
| min | minutes |
| ML | mediolateral |
| mRNA | messenger ribonucleic acid |
| ms | millisecond |
| MSC | mesenchymal stem cells |
| NaCl | sodium chloride |
| NG2 | neural/glial 2 |
| NuMa | nuclear mitotic apparatus protein |
| OB | olfactory bulb |
| PBS | phosphate buffered saline |
| PBS+ | PBS supplemented with 0.1% Triton™ X-100 and 10% donkey serum |
| PD | Parkinson's disease |
| PFA | paraformaldehyde |
| RME | reference memory error |
| SGZ | subgranular zone |
| sMSC | mesenchymal stem cell spheres |
| SN | substantia nigra |
| SVZ | subventricular zone |
| TB | tooth bar |
| TH | tyrosine hydroxylase |
| vWF | von Willebrandt Factor |
| WME | working memory error |

## 2. Summary

### *2.1. Abstract*

***Introduction*:** Adult mesenchymal stem cells (MSC) can be easily derived from many tissues, are immune privileged, and secrete factors important in modulating inflammation and neurogenesis. Consequently, they are ideal candidates for stimulating repair mechanisms in neurodegenerative and inflammatory disorders, such as Parkinson's disease (PD). This thesis characterises the development of MSC and their short- and long-term effects on motor and non-motor symptoms in a PD rat model. Another aim was to explore reliable long-term labelling of MSC to enable *in vivo* tracking. In addition, we compared the transplantation properties of three-dimensional MSC spheres (sMSC) with adherent MSC. These two culturing techniques differ in their proliferation and differentiation potentials, which might in turn determine the suitability of the transplant.

***Methods*:** In our first study, MSC/sMSC were transplanted as naive MSC, while the two later studies used MSC/sMSC transfected with green fluorescent protein. One week after lesioning with 6-hydroxydopamine, MSC/sMSC were transplanted into the substantia nigra. The animals were sacrificed three days (Study 2), three weeks (Study 1), and six months (Study 3) after transplantation. Motor behaviour was assessed with rotational behaviour and memory was tested with the 8-arm radial maze test (Studies 1 and 2). The first study focussed on hippocampal and the two follow-up studies primarily on subventricular neurogenesis. Glial fibrillary acidic protein (GFAP) and ionised calcium-binding adapter molecule 1-positive cells characterised the initial immunological response (Study 1), which was followed up by analyses of peripheral inflammatory factors (Study 3).

***Results*:** In both the short- and long-term, MSC/sMSC expressing endothelial markers were found in the arachnoid mater and around the lumen of blood vessels. At three days, no difference was seen between the transplant types: MSC and sMSC both improved subventricular neurogenesis, without affecting dopaminergic recovery. Three weeks after transplantation, MSC had improved motor behaviour, and induced striatal up-regulation of GFAP and brain-derived neurotrophic factor, while sMSC had exerted undesirable effects on hippocampal neurogenesis and memory. At six months, MSC had increased dopamine levels and neurogenesis, improved memory, and induced up-regulation of anti-inflammatory cytokines.

***Conclusion*:** MSC are superior to sMSC; making them more suitable candidates for transplantation. Hyposmia and memory decline in PD patients are the two main non-motor symptoms related to aberrant neurogenesis. Thus, cell transplants that can increase neurogenesis in the hippocampus and subventricular zone could represent a disease-modifying therapy, while also protecting against dopaminergic degeneration and modulating the systemic immune response.

## *2.2. Zusammenfassung*

***Einleitung*:** Adulte mesenchymale Stammzellen (MSC) können leicht aus vielen Gewebearten gewonnen werden, sind immunprivilegiert und sezernieren bedeutende Faktoren für die Entzündungsregulation sowie Regeneration. Dementsprechend können MSC in der Therapie neurodegenerativer Krankheiten, wie dem idiopathischen Parkinson Syndrom (IPS), optimal genutzt werden. Die vorliegende These untersuchte die MSC Entwicklung und deren Kurz- und Langzeiteffekte auf motorischer und nicht-motorischer Ebene im 6-Hydroxydopamin Rattenmodell der Parkinsonschen Erkrankung. Ferner sollte eine effiziente Art der Langzeit-MSC-Markierung untersucht werden, um eine zuverlässige *in vivo* Charakterisierung zu gewährleisten. Da MSC sowohl als dreidimensionale Sphären kultiviert werden, als auch als adhärente Zellen – was zu unterschieden in der Differenzierung und Proliferation führt – wurden adhärente und sphärische MSC (sMSC) in zwei der Studien gegenübergestellt.

***Methoden*:** Eine Woche nach der Läsion mit 6-Hydroxydopamin wurden die MSC/sMSC in die Substantia nigra transplantiert und die Ratten nach drei Tagen (Studie 2), drei Wochen (Studie 1), oder sechs Monaten (Studie 3) post mortem untersucht. Sowohl hippokampale, als auch subventrikuläre Neurogenese wurden ausgewertet. Motorische Symptome wurden mittels Amphetamin-induziertem Rotationsverhalten gemessen und Gedächtnisstörungen wurden mit dem 8-Arm Radial Maze Test überprüft (Studie 1 und 2). Die Entzündungsreaktion wurde initial durch die Anzahl saurer Gliafaserprotein- und Iba1-positiver Zellen gemessen (Studie 1) und später durch multiplexe Analysen systemischer inflammatorischer Faktoren erweitert (Studie 3).

***Ergebnisse*:** MSC/sMSC konnten kurz- und langfristig mit entothelialen Markern charakterisiert werden und wurden in der Nähe von Blutgefäßen und in der Arachnoidea mater encephali gefunden. Nach drei Tagen gab es keine Unterschiede zwischen den zwei Zelltransplantaten: beide stimulierten die subventrikuläre Neurogenese, ohne Effekte auf die dopaminergen Neurone. Nach drei Wochen bewirkten die MSC sowohl eine striatale Hochregulierung vom sauren Gliafaserprotein und vom neurotrophen Faktor *Brain-derived neurotrophic factor* (BDNF) als auch eine Verbesserung in der Motorik. sMSC hingegen beeinträchtigten die hippokampale Neurogenese und die Lern- und Gedächtnisfunktion. Nach sechs Monaten erhöhten die MSC den nigralen Dopamin-Spiegel und die subventrikuläre und hippokampale Neurogenese, verbesserten das Gedächtnis und steigerten systemische anti-inflammatorische Zytokine.

***Schlussfolgerung*:** MSC zeigten vorteilhaftere Effekte gegenüber sMSC und wirkten bis zu sechs Monaten neurotroph. Hyposmie und kognitive Defizite sind die Kardinalsymptome des nicht-motorischen Spektrums in IPS und stehen in Zusammenhang mit gestörter Neurogenese. Somit könnten Zelltransplantate mit modulierenden Effekten auf den Hippokampus und die subventrikuläre Zone eine krankheitsmodifizierende therapeutische Strategie darstellen und gleichzeitig vor dopaminerger Degeneration schützen.

### *2.3. Introduction*

Parkinson's disease (PD) is the most common neurodegenerative movement disorder, affecting about 1% of those older than 60 years [1]. Key pathological events are the progressive loss of dopaminergic neurons in the substantia nigra (SN), and the formation of α-synuclein-positive inclusions in cell bodies and neurites of nigral and olfactory bulb (OB) neurons (Lewy bodies) [2, 3], while neurogenesis decreases in the subgranular zone (SGZ) and the subventricular zone (SVZ)–OB axis [4]. Aberrant neurogenesis and progressive neuronal loss may cause hyposmia and cognitive decline in PD patients [4]. Such non-motor impairments precede the motor symptoms in PD [5] and may dominate the pathology, severely affecting the patients' quality of life [6]. Consequently, non-motor symptoms are a potential valuable early therapeutic target.

The prevailing treatment of PD with dopaminergic drugs such as L-DOPA, achieves only symptomatic relief. Conversely, transplanting embryonic or induced pluripotent stem cells might counteract the progressive degeneration, but such a treatment still faces many obstacles, including the risk of teratoma formation [7] and the spread of Lewy bodies in the transplanted allografts [8-10]. Modulation of the pro-inflammatory microenvironment, which is a potential cause of dopaminergic cell death in PD [11] is another recent aetiology-oriented treatment option. Mesenchymal stem cells (MSC) have the potential to influence this microenvironment, by releasing neurotrophic and anti-inflammatory factors [12]. Moreover, recent studies showed that MSC improve motor deficits and partially restore dopaminergic marker expression in the striatum and SN of Parkinsonian rats [13-18]. Beyond that, MSC have been employed in many disease models as well as in numerous clinical trials [19] including one addressing PD [20]. Furthermore, MSC have the advantage over embryonic or induced-pluripotent stem cells that they are easily harvested and expanded, are not subjected to ethical constraints, are immune-privileged and are non-tumorigenic upon transplantation [12]. In addition, MSC are able to migrate towards the regions of injury [21], which enables them to function as a targeted delivery system for trophic factors and cytokines. PD increases pro-inflammatory cytokines and depletes neurotrophins [22, 23], hence trophic and anti-inflammatory MSC transplants could support endogenous repair systems, as shown in a study conducted in 6-hydroxydopamine (6-OHDA) lesioned rats [14] and Parkinsonian mice [24]. In these, adult subventricular neurogenesis was increased following MSC transplantation, along with neuronal precursor cell differentiation in the SN [24]. Similar effects of MSC transplantation were seen in hippocampal neurogenesis [25]. Thus, neurogenic MSC might restore the altered neurogenesis in PD and eventually improve memory and olfaction, thereby providing a promising regenerative therapy for non-motor symptoms in PD.

Many recent studies attributed superior properties such as a greater differentiation potential and greater trophic and anti-inflammatory factor release to MSC assembled as spheres (sMSC) [26-29]. In addition, three-dimensional sMSC show more cell and matrix interactions than monolayer-cultured cells, while mimicking a more natural cell microenvironment. On the other hand, adherent MSC are anchorage-dependent and

therefore might interact and adapt differently *in vivo*. These differences in cell aggregation might account for the different effects seen *in vivo*, but they have not yet been compared in a PD model.

### *2.4. Objective*

To explore the most effective type of MSC transplant, our first research aim was to compare two MSC culture models (sMSC *versus* MSC) *in vitro* and *in vivo* (**1**). Many studies have indicated that MSC transplants gradually vanish [16, 25, 30-32], stressing the need for reliable long-term tracking. We established this using transfection and human-specific antigens (**2**). Proper tracking enables MSC to be systematically characterised in the short- and long-term, another major research aim of this study (**3**). The last aim of this thesis was to analyse how the effect of MSC was mediated with respect to motor and non-motor symptoms, inflammation and in particular with respect to neurogenesis (**4**) in a PD rat model.

### *2.5. Methodology*

All methods used in this thesis, including the cell culture experiments conducted by Manfred Roch (AG Prof. Dr. Dr. Klose, BCRT, supervisor Prof. Andreas Kurtz), are described thoroughly in the respective publications listed in the appendix. In this section, study designs and key methods are summarised.

#### *2.5.1. Animals*

The studies were carried out with the permission of the responsible local authorities (Landesamt für Gesundheit und Soziales, Berlin) and in accordance with EU Directive 2010/63/EU. Wistar rats (Harlan-Winkelmann, Borchen, Germany; 220–240 g) were kept on a 12-h light/dark cycle in a temperature- and humidity-controlled vivarium with free access to food and water. Suffering was minimised with appropriate anaesthetics. The rats were randomly assigned to the groups, and the investigators were blinded to the groups.

#### *2.5.2. In vivo design of Study 1*

Adult Wistar rats were given two stereotaxic injections of 2µl 6-OHDA (Sigma-Aldrich, Steinheim, Germany) or 2µl 0.9% sodium chloride (NaCl; *n*=10, sham injury group) into the left medial forebrain bundle (MFB). One week after lesioning, rotational behaviour in response to a d-amphetamine sulphate (Sigma-Aldrich, Steinheim, Germany) injection was tested. Thereafter, lesioned rats were evenly divided into three subgroups to receive either 5µl 0.9% NaCl (*n*=10, sham cell administration group), 5µl sMSC (*n*=10, sMSC group) or MSC infusions (*n*= 10, MSC group) ipsilateral in the SN. The four groups are referred to as "MSC", sMSC", "sham cell administration" and "sham injury". "On the day of transplantation and on the two subsequent days, the rats were given intraperitoneal (i.p.) injections of 5-bromo-2-deoxyuridine (BrdU;

50mg/kg; Sigma-Aldrich, Steinheim, Germany). Eight-arm radial maze memory testing was performed two weeks after transplantation. Hereafter, rotational behaviour was re-tested and compared to the pre-transplantation situation (Fig. 1a, thesis). One day later, all rats were transcardially perfused.

### *2.5.3. In vivo design of Study 2*

Adult Wistar rats were given two stereotaxic injections of 2µl 6-OHDA or 2µl 0.9% NaCl (*n*=10, sham injury group) into the left MFB. Thereafter, the 6-OHDA-lesioned group was evenly divided into three subgroups to receive either 5µl 0.9% NaCl (*n*=10, sham cell administration group), 5µl sMSC (*n*=10, sMSC group) or MSC (*n*=10, MSC group) ipsilateral in the SN. The four groups are referred to as "MSC", "sMSC", "sham cell administration" and "sham injury". On the day of transplantation and on the two subsequent days, the rats were given i.p. injections of BrdU (50mg/kg) and were transcardially perfused after three days of transplantation (Fig. 1b, thesis).

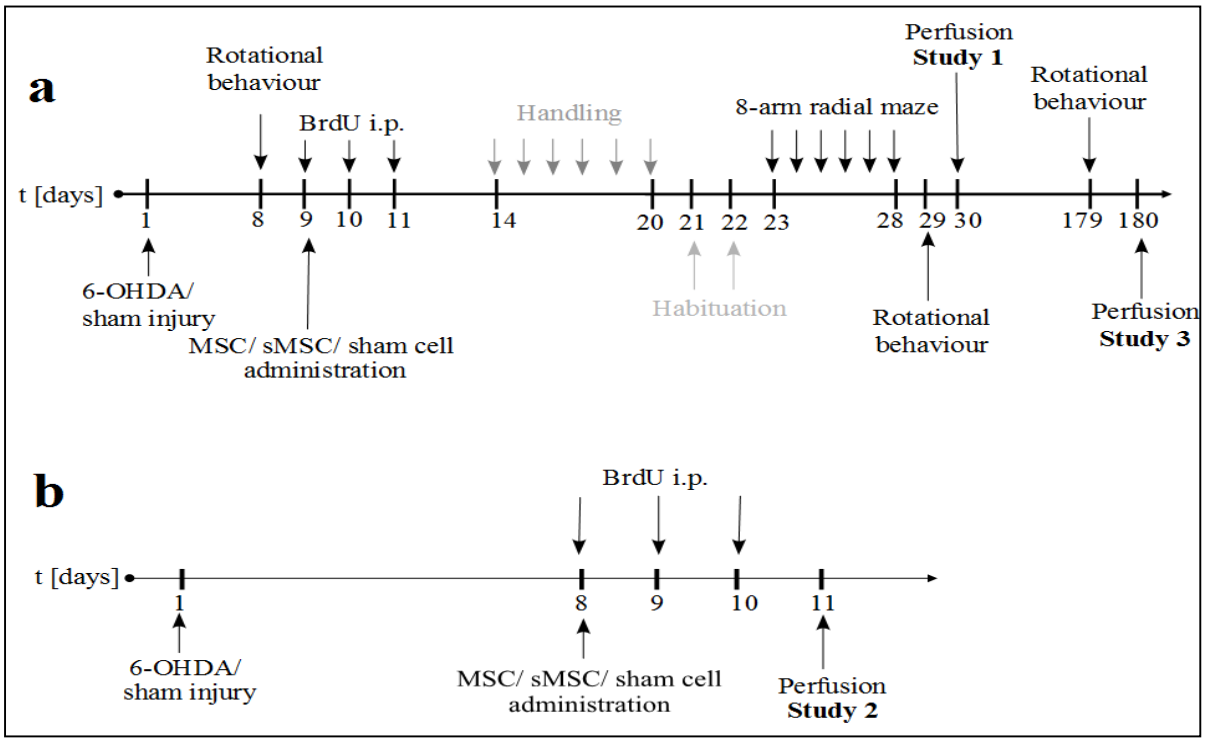


**Fig. 1** Timelines of experiments. Abbreviations: 6-OHDA, 6-hydroxydopamine; BrdU, 5-bromo-2-deoxyuridine; i.p., intraperitoneal; MSC, mesenchymal stem cell.

### *2.5.4. In vivo design of Study 3*

Adult male Wistar rats were given two stereotaxic injections of 2µl 6-OHDA or NaCl (*n*=7, sham injury group) into the left MFB. One week after lesioning, rotational behaviour in response to a d-amphetamine sulphate injection was tested. Thereafter, the lesioned animals were given either 5µl NaCl (*n*=7, 6-OHDA group) or

5µl MSC (*n*=14, AD-MSC group) ipsilateral in the SN. The three groups are referred to as "AD-MSC", "6-OHDA" and "sham". On the day of transplantation and on the two subsequent days, the rats were given i.p. injections of BrdU (50mg/kg). The animals were tested with the 8-arm radial maze two weeks after transplantation and rotational behaviour was retested after three weeks and one day before perfusion after six months (180 days; Fig. 1a, thesis). Due to the divergent labelling in Study 3 ('AD-*MSC'*, '6-OHDA', 'sham' *versus* 'MSC', 'sham cell administration', 'sham injury'*),* we used the label 'MSC' to refer to 'AD-MSC', the label 'sham cell administration' to refer to '6-OHDA', and 'sham injury' to refer to 'sham' throughout this thesis.

### *2.5.5. Immunohistochemistry*

The rats were anaesthetised with chloral hydrate and transcardially perfused with cold 0.1M phosphate buffered saline (PBS; Carl Roth, Karlsruhe, Germany) followed by 2% paraformaldehyde (PFA; Carl Roth, Karlsruhe, Germany) diluted in 0.1M PBS. Brains were post-fixed for 15min in 2% PFA followed by cryoprotection with 30% sucrose (Carl Roth, Karlsruhe, Germany) for 48h. Brains were frozen in chilled 2-methylbutane (Sigma-Aldrich, Steinheim, Germany), stored at -80°C, and then cut into 40µm coronal sections. For BrdU staining, DNA was denatured in 2N hydrochloric acid for 30min at 37°C and then rinsed in 0.1M borate buffer (pH 8.5) for 10min. For immunohistochemical analyses, sections were pre-treated with 0.6% hydrogen peroxide and incubated with the primary antibody (AB) overnight at 4°C and with the secondary AB for 2h in PBS supplemented with 0.1% Triton™ X-100 (Sigma-Aldrich, Steinheim, Germany) and 10% donkey serum (PBS+) at room temperature. The VECTASTAIN® ABC kit (Vector Laboratories, Burlingame, USA) was combined with the chromogen 3,3'-diaminobenzidine (DAB; Sigma-Aldrich, Steinheim, Germany). For immunofluorescence analyses, sections were incubated with the primary AB over night at 4°C and with the secondary AB for 4h in PBS+ at room temperature. Sections were then counterstained with 4',6-diamidino-2-phenylindole (DAPI; 1:1000, Ferak, Berlin, Germany) and coverslipped in Protaqs®Clear (Quartett, Berlin, Germany). Histological and cytological staining was repeated at least three times for evaluation.

*Primary antibodies:*

Anti-brain-derived neurotrophic factor (BDNF; 1:50, Santa Cruz Biotechnologies, Heidelberg, Germany), anti-BrdU (1:500, AbDSerotec, Puchheim, Germany), anti-cluster of differentiation (CD) 31 (CD31; 1:200, Abcam, Cambridge, UK), anti-CD34 (1:200, Santa Cruz Biotechnologies, Heidelberg, Germany), anti-CD146 (1:200, Santa Cruz Biotechnologies, Heidelberg, Germany), anti-CD44 (1:50, Tonbo Biosciences, San Diego, USA), anti-glial cell line-derived neurotrophic factor (GDNF; 1:50, Abcam, Cambridge, UK), anti-glial fibrillary acidic protein (GFAP; 1:1000, Sigma-Aldrich, Steinheim, Germany), anti-green fluorescent protein (GFP; 1:1000, Acris, Herford, Germany), anti-ionised calcium-binding adapter molecule 1 (iba1; 1:1000, Wako Pure

Chemical Industries, Japan), anti-HuD (1:50, Santa Cruz Biotechnologies, Heidelberg, Germany), anti-human mitochondria (HuMi; 1:100, Millipore, Temecula, USA), anti-microtubule associated protein (MAP2b; 1:200, NOVUS Biologicals, Littleton, USA), anti-major histocompatibility complex class I (MHC-I; 1:250, Abcam, Cambridge, UK), anti-neural/glial 2 (NG2; 1:200, Millipore, Temecula, USA), anti-nuclear mitotic apparatus protein (NuMA; 1:50, Abcam, Cambridge, UK), anti-S100β (1:1000, Sigma-Aldrich, Steinheim, Germany), anti-tyrosine hydroxylase (TH; 1:100, Sigma-Aldrich, Steinheim, Germany), anti-vimentin (1:100, Abcam, Cambridge, UK), and anti-von Willebrandt Factor (vWF; 1:200, Millipore, Temecula, USA).

*Secondary antibodies:*

Anti-rhodamine X (1: 250, Dianova, Hamburg, Germany), anti-Alexa647 (1:1000, Dianova, Hamburg, Germany), anti-Alexa488 (1:1000, Life Technologies, Carlsbad, USA), and anti-biotin (1:250, Jacksons Immunoresearch, West Baltimore, USA).

### *2.5.6. Inducing parkinsonism via 6-OHDA lesioning*

To achieve an end-stage PD model [33], the left MFB was fully lesioned. The rats were anaesthetised with sodium pentobarbital (60mg/kg; Sigma-Aldrich, Steinheim, Germany) and positioned on the stereotaxic apparatus (David Kopf Instruments, Tujunga, USA). After making two holes in the skull with a dental drill, two stereotaxic injections of 2µl 6-OHDA (6.5µg diluted in 0.9% NaCl and 0.1% ascorbic acid) were applied using a 26s gauge Hamilton microsyringe (model 801). The cannula was left in place for 5min after the injection to optimise toxin diffusion. The following bregma coordinates according to Paxinos and Watson [34] were chosen: tooth bar (TB): -2.4, anterior–posterior (AP): -4.4, mediolateral (ML): +1.2, dorsoventral (DV): -7.8; and the following interaural coordinates: TB: +3.4, AP: -4.0, ML: +0.8 and DV: -8.

### *2.5.7. Intranigral MSC transplantation into the Parkinsonian rat brain*

The surgical procedure was the same as described for the 6-OHDA infusion. After making a hole in the skull with a dental drill, one stereotactic injection of $3x10^5$ cells in 5µl PBS was infused over 2min through a 22s gauge needle with a Hamilton microsyringe (model 1702) that was retracted after 5min. The following bregma coordinates were used for the intranigral infusion: TB: -3.3, AP: -5.3, ML: 2.4 and DV: -7.4 (see supplementary Fig. 1, Study 1), according to Paxino and Watson [34].

### *2.5.8. Memory testing with the eight-arm radial maze test (Studies 1 and 3)*

Based on a previous protocol 35, the 8-arm radial maze had an octagonal centre platform with eight radial arms and a food cup at the far end of each arm. The experimental room contained visible cues to allow spatial orientation. Food restriction (2h per day with water ad libitum) started three days before the experiment and

continued throughout the test, while body weight was monitored daily. During the first two days of the eight-day testing period the rats were habituated to the maze by placing peanuts in every arm and leaving the animals in the maze for 10min. During the memory test phase, only the arms directed towards the cues were baited and the rats remained in the maze until all three rewards had been eaten or until 10min had elapsed. The first entry into a nonbaited arm was scored as a reference memory error (RME) and any arm re-entry (in a formerly baited or nonbaited arm) was scored as a working memory error (WME). The test was conducted from day 21 to day 28 (Fig. 1a, thesis).

*2.5.9. Motor function measured with d-amphetamine induced rotation (Studies 1 and 3)*

The rotational response to d-amphetamine is a functional measure of unilateral dopaminergic denervation [35]. The rats were injected intraperitoneally with d-amphetamine sulphate (2.725mg/kg dissolved in 0.9 % NaCl; Sigma-Aldrich, Steinheim, Germany) and placed into automated rotameter bowls (TSE Systems, Bad Homburg, Germany). The numbers of turns exceeding 180° were recorded during a 90min period that started 15min after injection.

*2.5.10. Statistical analyses*

Normality and variance homogeneity were tested using the Kolmogorov-Smirnov and Levene's test, respectively. Data were presented as mean and standard error of the mean and the significance level was set at $p$=0.05. Whenever normality could be assumed, group comparisons were performed with t-tests, analysis of variance (ANOVA), repeated measurements ANOVA, or multivariate ANOVA, followed by Tukey-HSD (equal variances), LSD (equal variances), and Tamhanes (unequal variances) post hoc comparisons, using SPSS (IBM, version 21). Correlations were analysed with Spearman's rank correlation coefficient (non-parametric). Non-normally distributed data were log transformed [log10; multiplex and SVZ BrdU analyses of Study 2].

## *2.6.* Results

This section provides a short, comprehensive summary of the published results. The detailed results of the experiments are described in the publications listed in the appendix.

*2.6.1. Study 1: MSC improve motor function and increase BDNF in a rat model of PD*

Differences between sMSC and MSC were found *in vitro*: sMSC up-regulated genes of the nerve-growth-factor-receptor [$n$=3, $F$(1,2)=751.09, $p$<0.005], GDNF [$n$=2, $F$(1,2)=9572.24, $p$<0.001], and the cell-adhesion molecule CD49b [$n$=3, $F$(1,2)=2171.40, $p$<0.001] more than MSC. MSC up-regulated the bone-

related SPARC gene more than sMSC [$n$=4, $F$(1,6)=30.99, $p$<0.01]. Non-neuronal growth factors (c-myc, BGLAP, RUNX2) showed no difference in gene expression ($p$>0.05; Fig. 5).

Twenty-one days after transplantation, viable MSC/sMSC were detected by NuMa staining (Fig. 13). Although MSC grafts improved motor function [$F$(3,41)=25.192, $p$<0.001; post hoc: $p$<0.05], they were unable to reverse the profound decrease in ipsilateral TH-positive (TH+) neurons: all lesioned animals had fewer TH+ cells in the lesioned hemisphere than in the non-lesioned hemisphere [$F$(3,32)=24,737, $p$<0.001; post hoc: $p$<0.05] and compared to sham injury ($p$<0.05; Fig. 6). However, MSC enhanced contralateral TH+ cell numbers more than sMSC ($p$<0.05). In line with this, MSC increased contralateral BDNF mRNA levels, a neurotrophin important for the survival of dopaminergic neurons [36, 37], to a greater extent than sMSC [$F$(1,52)=4.097, $p$<0.05; post hoc: $p$<0.005] or sham cell administration ($p$<0.01; Fig. 7).

MSC/sMSC increased the ipsilateral inflammatory response as measured by the amount of iba1-positive (iba1+) microglia [$F$(3,31)=85.712, $p$<0.001] compared to sham cell administration ($p$<0.01) and sham injury ($p$<0.01). Yet, treatment with sMSC still lead to higher numbers of iba1+ microglia than treatment with MSC ($p$<0.001; Fig. 11). With regard to striatal astroglia activation, all 6-OHDA-lesioned animals had more GFAP-positive (GFAP+) cells in the ipsilateral hemisphere than in the contralateral hemisphere [$F$(3,32)=4.720, $p$<0.01; post hoc: $p$<0.01], and MSC grafted animals had more GFAP+ cells than those with sham cell administration or sham injury ($p$<0.05, Fig. 8a). Similarly, MSC treatment increased nigral GFAP mRNA levels more than all other treatments [$F$(3,52)=6.049, $p$<0.005; post hoc: $p$<0.05], and GFAP mRNA levels were higher in the ipsilateral than in the contralateral hemisphere ($p$<0.005, Fig. 8b). On the other hand, sMSC decreased BrdU-positive (BrdU+) cells in the SGZ compared to MSC [$F$(3,32)=3.841, $p$<0.05; post hoc: $p$<0.01; Fig. 10]. In line with this, only sMSC worsened working memory, as shown by increased WME during the 8-arm radial maze test [$F$(15,205)=2.649, $p$<0.001], on the first day of testing ($p$<0.05; Fig. 12a).

*2.6.2. Study 2: MSC increase endogenous neurogenesis in the SVZ acutely following 6-OHDA lesioning*

Given the profound differences between MSC and sMSC that were discovered in our first proof-of-principle study, the intention of the second study was to provide a detailed phenotypical *in vitro* and *in vivo* analysis, while extending the statistical analyses. In line with our first study, cultured sMSC up-regulated neuroepithelial [Nestin, CD146, and CD31; $n$=3, $F$(1,4)=67.88, $p$<0.01, partial $\eta^2$=0.94], glial [NG2, CNPase; $n$=3, $F$(1,4)=79.27,$p$<0.005, partial $\eta^2$=0.95], and pluripotency genes [NANOG and KLF4; $n$=4, $F$(1,6)=9.20, $p$<0.05, partial $\eta^2$=0.51; Fig. 3e] more than MSC. Although the *in vitro* antigen expression was very similar for both MSC types (Table II), only MSC expressed the trophic factor BDNF (Fig. 4a) and CD31 (Fig. 4f). All MSC/sMSC expressed the mesenchymal cytoskeletal protein vimentin (Fig. 4b), the endothelial marker vWF (Fig. 4c), the neural precursor and multipotent antigen SOX2 (Fig. 4d), and the astrocytic

protein S100β (Fig. 4e). *In vivo*, MSC continued to express SOX2 (Fig. 7a), S100β (Fig. 7b, 7c and 7e), BDNF (Fig. 7d, Fig. 8a), and vWF (Fig. 8b, Fig. 10c and 10h). In addition, MSC expressed the neuronal marker MAP2b (Fig. 7e), the pericytic marker CD146 (Fig. 10g), the hematopoietic progenitor antigen CD34 (Fig. 10d), while some MSC expressed the proliferative antigen BrdU (Fig. 8c). As opposed to the results of our first study, MSC were localised not only in the surround of the transplant, but also in and around blood vessels (Fig. 8d and 8e, Fig. 9a, and 9e, Fig. 10a-d), and in the nigral arachnoid mater (AM; Fig. 7a-e, Fig. 8a, Fig. 8c, Fig. 9b). Furthermore, all transplant sites showed many vWF-, CD34-, and CD31-positve cells around and below the transplant (Fig. 8b), indicating a possible neovascularisation.

In contrast to the results of our first study, we did not find any differences between MSC- and sMSC-treated rats *in vivo*. Therefore, and to enhance the power of the statistical analyses, we decided to summarize the findings for both cell types as a single group that we named 'MSC'.

Another addition to our first study was the assessment of neurogenesis in the SVZ, in which precursor cell proliferation depends on dopaminergic innervations [38, 39], implying a potential role of SVZ precursor proliferation in the modulation of dopamine. As described in the literature [40], all 6-OHDA-lesioned animals ($n$=19, $M$=10407±4793) showed increased total numbers of BrdU+ proliferating cells in the SVZ [$F(1,23)=4.40$, $p<0.05$, partial $\eta^2=0.16$] compared to those with sham injury ($n$=6, $M$=5844±4089). In agreement with the data of the first study, assessing neurogenesis in the SGZ, we found that MSC ($n$=17, $M$=8.9±2%) increased neuronal precursor proliferation in the SVZ to a greater extent [quantified by BrdU and HuD double-positive cells (BrdU+/HuD+)] than in all other groups [$F(2, 27)=43.45$, $p<0.001$, partial $\eta^2=0.76$; post hoc: $p<0.001$], while sham cell transplantation ($n$=4, $M$=1.7±0.3%) led to fewer BrdU+/HuD+ cells than sham injury ($n$=9, $M$=4.7±0.6%; $p<0.001$) (Fig. 5a-d, Fig. 6).

Furthermore, as the first study tracked MSC/sMSC with only one human-specific AB and detected cells only in the transplantation area, a major follow-up aim was to label MSC with GFP, in addition to using several different antibodies. Although *in vitro* GFP expression was stable until senescence (data not shown), some MSC stopped expressing GFP *in vivo* (Fig. 2a-d, thesis). Yet all GFP-labelled MSC expressed human-specific vimentin and HuMi (see supplementary Fig. 2); in addition human-specific CD44 (Fig. 2e-h – thesis) and MHC-I (quantification of MSC survival) were used to verify MSC survival and to establish valid long-term markers.

Another extension of our initial study was the quantification of MSC survival. We showed that about 31% ($n$=2, $M$=92268±2399) survived in the surround of the transplant. These numbers are only approximations of an exemplary nature due to the small number of animals examined, and considering that many MSC localised in the cerebral AM, which often only partially remained at the brain surface.

In accordance with the first study, unilateral 6-OHDA lesioning significantly decreased the number of nigral TH+ neurons compared to animals with sham injury (sham cell administration: $M$=49±4%, sham

injury: *M*=97±1%, MSC: *M*=56±17%; *F*(2,37)=29.77, *p*<0.001, partial $\eta^2$=0.62; Fig. 11), indicating a lack of TH recovery by MSC treatment three days after transplantation.

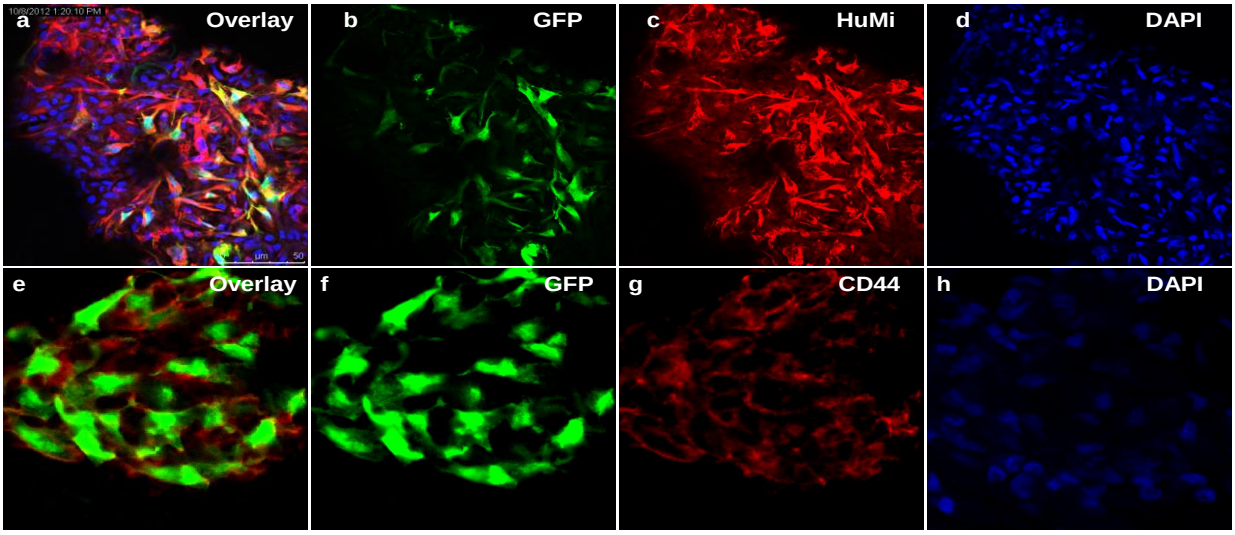


**Fig. 2** GFP labelling *versus* HuMi antigen expression by MSC *in vivo* (**a**-**d**). GFP-positive MSC co-localising human-specific CD44 *in vivo* (**e**-**h**). Abbreviations: DAPI, 4',6-diamidino-2-phenylindole, GFP, green-fluorescent protein; HuMi, anti-human mitochondria; MSC, mesenchymal stem cell.

### *2.6.3. Study 3: MSC induce long-term neurogenic and anti-inflammatory effects and improve cognitive, but not motor performance in a rat model of PD*

As we found no differences between MSC and sMSC treatment in hemiparkinsonian rats in the short-term, and since our first study indicated a favourable effect of MSC, the last study was intended to assess only MSC treatment. Furthermore, as we established proper short-term tracking and characterization of MSC/sMSC, the last study was designed as a longer-term follow-up of these findings, i.e. six months post transplantation, while including the same battery of behavioural tests as for the first study.

In line with the results of the second study, quantifying of BrdU+/HuD+ cells showed more newly generated surviving neurons after MSC treatment (*n*=14, *M*=15±7.3%) or sham injury (*n*=7, *M*=13±6.4%) than after sham cell administration [*n*=7, *M*=4.2±2.2%; *F*(2,25)=7.17, *p*<0.005, partial $\eta^2$=0.36; post hoc: *p*<0.005 and *p*<0.05; Fig. 3B, Fig. 5a-c, Table 1], while sham injury and MSC groups did not differ. In line with this, MSC treatment and sham injury led to more surviving BrdU+ cells in the SGZ of the hippocampus than did sham cell administration [*F*(2,16)=14.7, *p*<0.001, partial $\eta^2$=0.65, post hoc: *p*<0.05; Fig. 3A, Fig. 6a-d, Table 2]. Contrary to the findings of the second study, there were more ipsilateral BrdU+ cells in the SVZ in sham injured animals (*n*=7, *M*=2472±1327) than in sham cell transplanted animals [*n*=7, *M*=1204±403; *F*(2,25)=3.73, *p*<0.05, partial $\eta^2$=0.23, post hoc: *p*<0.05]. The numbers of BrdU+ cells did not differ between

MSC transplanted animals ($n$=14, $M$=1717±916) or those with sham injury or sham cell administration (Fig. 3A, Fig. 4a-d, Table 1).

Contrary to the results of the first and second studies, MSC treatment restored ipsilateral TH+ cell numbers, compared to sham cell administration [$F(2,25)=26.61$, $p<0.001$, partial $\eta^2=0.68$, post hoc: $p<0.001$]. However, sham injury controls showed still higher TH+ cell numbers than MSC-treated animals ($p<0.001$) and those with sham cell administration ($p<0.001$) (Fig. 9a-d, Table 3). Furthermore, the amount of subventricular BrdU+/HuD+ neurons correlated positively ($n$=28, $\rho$=0.40, $p<0.05$) with the number of TH+ neurons in all groups, indicating a relationship between dopaminergic neurons and subventricular neurogenesis, as described in the literature [41]. No BrdU or TH double-positive cells were seen in any of the groups, implying an absence of dopaminergic neurogenesis six months after transplantation.

Six months post transplantation, all MSC had lost their GFP expression, but were identified by robust human-specific antigen expression, as established in the short-term in the second study, i.e. with HuMi, MHC-I, and CD44 (Fig. 7a-e and Fig. 8). Similar to our short-term results, most MSC were found in the nigral AM, expressing CD34 (Fig. 8d and 8h), vWF (Fig. 8i), SOX2 (Fig. 8n), BDNF (Fig. 8r), and NG2 (Fig. 8w), a marker for polydendrocytes and pericytes. Neuronal ABs were not expressed by MSC (Fig. 8s and 8x, supplementary Table 2).

Extending on the assessments of inflammation in the first study, the third study examined the peripheral inflammatory response and culture media using multiplexed analyses of 22 pro- and anti-inflammatory factors. Only vascular endothelial growth factor, a potent factor for vasculogenesis and angiogenesis, was elevated in MSC media compared to unconditioned media. Serum analyses indicated that levels of erythropoietin (EPO), a growth factor for hematopoietic progenitor cells, were higher in the serum of MSC-treated [$F(2,22)=6.55$, $p<0.05$, partial $\eta^2=0.37$, post hoc: $p<0.005$] and sham injured animals ($p<0.05$) compared to those with sham cell administration. Furthermore, levels of the anti-inflammatory factor interleukin (IL)-10 were higher after MSC treatment [$F(2,24)=6.45$, $p<0.01$, partial $\eta^2=0.35$, post hoc: $p<0.05$] or sham injury ($p<0.05$), than after to sham cell administration. IL-2 levels were also elevated by MSC treatment compared to sham cell administration [$F(2,18)=5.00$, $p<0.05$, partial $\eta^2=0.36$, post hoc: $p<0.05$]. Levels of the anti-inflammatory factor IL-4 [42] were also increased more by MSC treatment [$F(2,23)=7.49$, $p<0.005$, partial $\eta^2=0.39$, post hoc: $p<0.005$] and sham injury ($p<0.05$), than by sham cell administration (Fig. 2, supplementary Table 3). In all analyses, sham injured and MSC-treated animals did not differ.

All groups improved their working [$F(5,21)=8.79$, $p<0.001$, partial $\eta^2=0.68$] and reference memory [$F(5,21)=8.73$, $p<0.001$, partial $\eta^2=0.68$] over time, however, sham cell administration caused impairments in working memory compared to sham injured controls (all test days except the third) and compared to MSC (from the fourth day of testing onwards) indicating that MSC transplantation improved working memory performance in lesioned animals (Fig. 10A, Table 4).

Contrary to the results of our first study, MSC treatment did not improve rotational behaviour performance: sham injured animals exhibited fewer rotations compared to MSC [$F(2,23)=4.89$, $p<0.02$, partial $\eta^2=0.30$; post hoc: $p<0.05$] and sham cell administration ($p<0.01$; Fig. 10B).

### *2.7.* Discussion

The PD literature concerning the ideal MSC transplant type and concerning suitable tracking of MSC that would enable a long-term phenotypic characterization is inconclusive. Therefore, this thesis assessed the most appropriate transplant type (**1**), established reliable long-term tracking of MSC (**2**), characterised MSC/sMSC development (**3**), and analysed how their effect is mediated based on motor and non-motor symptoms, inflammation and neurogenesis (**4**) in a PD rat model.

**(1)** Our first proof-of-principle study revealed that MSC/sMSC survive up to three weeks in the lesioned brain, but only MSC exerted favourable effects on motor and memory function, on contralateral TH-neuron survival, and on BDNF mRNA expression. On the contrary, sMSC grafts reduced hippocampal progenitors and contralateral TH+ cells, and increased microglia activation. These data indicate that the neurotrophic and protective effects of MSC strongly depend on the cell aggregation status – favouring monolayer-cultured MSC *in vivo*. Although, our second study found no *in vivo* differences between MSC and sMSC in the short-term, we observed that at three weeks after grafting MSC were neurotrophic but sMSC were not, leading to a temporary decrease in neurogenesis and memory function of sMSC-treated rats. In line with previous studies, which showed enhanced (trans) differentiation of sMSC *in vitro* [28, 43, 44], we found that sMSC up-regulated neuronal factors, but only MSC expressed BDNF antigens *in vitro*. We consider BDNF expression an important prerequisite, given that loss of nigral BDNF expression down-regulates the dopaminergic phenotype and ultimately leads to dopaminergic cell death in rats [37]. Thus, even though sMSC show more differentiation potential *in vitro*, MSC represent the ideal transplant, considering their superior trophic potential *in vitro*, and given their more beneficial and homogeneous effects *in vivo*.

**(2)** The *in vivo* tracking of MSC has often been an unresolved issue [16, 25, 30-32], nevertheless, we found viable MSC/sMSC after three days (Study 2), three weeks (Study 1), and six months (Study 3) at the transplantation site, around blood vessel lumina, and in the cerebral AM. Already after three days, some MSC/sMSC stopped expressing GFP. This silencing of GFP gene expression continued until six months post transplantation, at which time all MSC had stopped expressing GFP. The fact that MSC had stopped expressing GFP *in vivo* but continued expressing GFP until senescence *in vitro* most likely reflects changes in the epigenetic state of the transgene. Yet, at all time points, MSC were robustly stainable with several human-specific antigens.

**(3)** In the short- and long-term (three days and six months), MSC expressed endothelial, pericytic, glial, and neurotrophic antigens, while proliferative and neuronal markers were only expressed after three days. The proximity of MSC to blood vessels at all time points resembles the location of resident MSC [45] and indicates that externally applied MSC also gather in perivascular niches. Furthermore, adipose-derived MSC are possibly derived from the adventitia of larger vessels, *i.e.* from pericytes and endothelial cells [46, 47] which possibly maintain their endothelial phenotype and perivascular location.

**(4)** A reversal of lesion-induced motor deficits by MSC were seen only three weeks after transplantation, and these did not correlate with the number of TH+ neurons. After six months, MSC had restored the lesion-induced TH+ cell decline, but had not improved motor function. The outcomes of the rotational performance tests, however, must be interpreted with caution as they reflect only few Parkinsonian symptoms in the 6-OHDA model and show only low external validity [48, 49]. In addition, no linear correlation exists between rotometry and dopamine recovery [50]. This raises the possibility that rotometry might not cover the improvement induced by MSC. A study currently being conducted in our lab assesses motor behaviour with the cylinder test; so far the results indicate a more sensitive evaluation, i.e. more significant differences between MSC and 6-OHDA-lesioned animals.

MSC mainly exerted effects on non-motor and paracrine systems, as evidenced by improved working memory, and increased neurogenesis and anti-inflammatory factors. Only two previous studies examined subventricular neurogenesis in response to MSC treatment in PD [14, 24], but both only examined short-term effects (about 4 weeks). Our results suggest that MSC treatment increases neuronal precursor proliferation in the SVZ as early as after three days (Study 2) and supports their survival in the SVZ and the dentate gyrus over six months (Study 3). Furthermore, neurogenesis in the SVZ correlated with TH+ cell recovery (Study 3), confirming a relationship between dopaminergic innervation and neurogenesis. Hence, we assume that the effects of MSC are mediated through the modulation of neurogenesis, probably by the release and expression of BDNF and other trophic factors and cytokines. This is in line with the results of Study 3 that showed that MSC treatment normalised several anti-inflammatory and neurogenic factors, e.g. EPO, an anti-inflammatory growth factor that stimulates angiogenesis and neurogenesis [51, 52], and the anti-inflammatory IL-10, which is involved in the modulation of adult neurogenesis [53, 54].

This is the first study to investigate motor and non-motor effects of MSC and their long-term cerebral survival in a PD model. Only one previous study detected MSC after 4.5 months in the Parkinsonian brain [15]. However, in that study the MSC were delivered intranasally and cognitive effects were not assessed. Furthermore, no previous study has showed that cerebrally transplanted MSC express endothelial markers and localise around blood vessel lumina in Parkinsonian animals. Only one extracerebral study found that after six months intravenously infused MSC express vWF and integrate into the vasculature of the rat

myocardium as endothelial cells [55]. This endothelial MSC phenotype might be due to their origin as discussed in **(3)** of the discussion, but it might also be an attempt of MSC to restore the reorganisation of the vasculature that is induced by injecting 6-OHDA [56]. In addition, MSC might associate with blood vessels given their resemblance to the (peri)vascular niche [57] in which stem cells, including resident MSC, reside, and where neurogenesis is supported and reparative mechanisms are initiated. In line with the latter, we showed for the first time that MSC localise in the AM – a potential new stem cell niche [58, 59] that harbours progenitor cells with neuronal differentiation potential [58, 60, 61]. The AM contains trophic and chemotactic factors [59, 62] and supports precursor cell migration in response to injury [58, 61]. The web-like structure of the AM could conceivably be an ideal milieu for anchorage-dependent MSC. Furthermore, leptomeningeal cells, the native cells of the AM, resemble MSC morphologically and are also of mesenchymal embryonic origin, which possibly creates a supportive MSC microenvironment. And above all, the AM contains blood vessels and cerebrospinal fluid, creating a perivascular or stem cell niche-like microenvironment. We have thus identified the perivascular niche and the AM-stem cell niche as critical environments for *in vivo* MSC maintenance. Whether these support or instruct MSC in its contribution to tissue homeostasis and repair requires further investigation.

*This thesis has the following limitations:*

(1) We transfected MSC with GFP, given the low host immunogenicity [63] and the ease of demonstrating cell viability through signal detection. Yet, transfection often results in low and discontinuous transgene expression and gene silencing [63] , as seen in our and other stem cell studies [64-66]. Other labelling techniques have other disadvantages: vital dyes suffer from signal loss due to cell division [67, 68], and imaging techniques e.g. magnetic resonance imaging and positron emission tomography do not permit single-cell resolution [69]. Intravital two-photon microscopy allows single-cell resolution, but one cannot observe deeper brain areas with it. Hence, most tracking available techniques are unsuitable for the broad assessment of single migrating cells. Future studies should develop vectors that are more resistant to gene silencing or use human-specific ABs to track grafted MSC throughout the brain over a long-term period.

(2) We, as well as others in the field [13-15, 24] have not found evidence of dopaminergic transdifferentiation, but instead predominantly paracrine MSC effects. A striatal lesion would have therefore been more suitable, given that injecting striatal trophic factor protected nigrostriatal function and histology in the 6-OHDA model, while injection into the SN did not [70, 71]. Our results prove that nigral MSC transplantation provides functional and histological benefits; but improvement might have been greater with striatal transplantation.

(3) Another question concerning our study design is why we used allogeneic MSC instead of autologous MSC. The complex feedback system of immunomodulatory responses implies that differences between host and donor MSC could lead to different anti-inflammatory outcomes [72]. The primary reason for choosing human

MSC was the proximity to human studies. Besides, allogeneic MSC are approved for treating patients with graft-versus-host-disease in New Zealand and Canada [73]. Yet it would have been of great interest to compare autologous with allogeneic MSC in our model. Especially considering the differences in immunomodulation of different MSC sources: some showed no difference between transendocardial injections of autologous and allogeneic MSC [74] while others showed increased inflammatory responses after intra-articular injection of allogeneic or xenogenic MSC compared to autologous MSC [75].

(4) Inducing Parkinsonism with the catecholaminergic neurotoxin 6-OHDA, only affects one brain hemisphere and does not reproduce all PD associated symptoms. Contemporary models can combine genetic alterations and neurotoxin exposure and might therefore be a better representation of the human condition. We chose this model based on pre-examinations conducted by our group and because it allows for intra-individual comparisons due to the availability of a control hemisphere.

(5) The 8-arm radial maze test is unable to assess some of the memory deficits seen in PD patients. Non-demented PD patients show impairments in episodic memory [76], whereas the 8-arm-radial-maze test measures semantic memory and reference memory [77]. We therefore probably did not assess the predominant type of memory impairment in PD patients. Furthermore, whether episodic memory exists and can be measured in animals is still a point of debate [78, 79]. This brings into question what type of memory we and other studies are actually evaluating.

In this thesis we showed that, contrary to previous *in vitro* reports, sMSC are not an ideal graft in PD. MSC transplants, on the other hand, improved motor symptoms after six months and non-motor symptoms at all time-points. These effects were possibly mediated by the stimulation of neurogenesis and neurogenic factors. Future studies will have to unravel how MSC increase neurogenesis and test whether the application of mediating factors, such as IL-10 and EPO by themselves could give similar results, eventually enabling the external regulation of neurogenesis. Given that the key non-motor symptoms in PD, i.e. hyposmia and memory impairment, are related to abnormal neurogenesis and affect patients at an early stage, cell transplants with modulatory effects on the hippocampus and SVZ could provide a disease-modifying therapeutic strategy, while also delaying dopaminergic degeneration.

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## 3. Affidavit

I, Anne Schwerk certify under penalty of perjury by my own signature that I have submitted the thesis on the topic “Mesenchymal stem cell characterization and effects on motor and non-motor symptoms in a Parkinson’s disease rat model”. I wrote this thesis independently and without assistance from third parties, I used no other aids than the listed sources and resources.

All points based literally or in spirit on publications or presentations of other authors are, as such, in proper citations (see "uniform requirements for manuscripts (URM)" the ICMJE www.icmje.org) indicated. The sections on methodology (in particular practical work, laboratory requirements, statistical processing) and results (in particular images, graphics and tables) correspond to the URM (s.o) and are answered by me. My contributions in the selected publications for this dissertation correspond to those that are specified in the following joint declaration with the responsible person and supervisor. All publications resulting from this thesis and which I am author of correspond to the URM (see above) and I am solely responsible.

The importance of this affidavit and the criminal consequences of a false affidavit (section 156,161 of the Criminal Code) are known to me and I understand the rights and responsibilities stated therein.

Date _____ Signature _____

**Declaration of any eventual publications**

Anne Schwerk had the following share in the following publications:

**Publication 1:**
Berg J, Roch M, Altschüler J, Winter C, **Schwerk A**, Kurtz A, Steiner B. Human adipose-Derived Mesenchymal Stem Cells Improve Motor Functions and are Neuroprotective in the 6-Hydroxydopamine-Rat Model for Parkinson's Disease when Cultured in Monolayer Cultures but Suppress Hippocampal Neurogenesis and Hippocampal Memory Function when Cultured in Spheroids. *Stem Cell Rev*. 2015; 11(1):133-49.
**IF: 3.2**
Contribution in detail: 20%. Partly performed behavioural experiments, executed and analysed histo- and cytostaining, performed statistical analyses, and revised the manuscript.

**Publication 2:**
**Schwerk A**, Altschüler J, Roch M, Gossen M, Winter C, Berg J, Kurtz A, Steiner B. Human adipose-derived mesenchymal stromal cells increase endogenous neurogenesis in the rat subventricular zone acutely after 6-hydroxydopamine lesioning. *Cytotherapy*. 2015;17(2):199-214.
**IF: 3.1 (2014)**
Contribution in detail: 80%. Partly designed the study, performed the operations, executed and analysed histo- and cytostaining, performed stereological and non-stereological cell counting, performed statistical analyses and data interpretation, drafted and revised the manuscript, and partly did the correspondence to

the reviewers.

**Publication 3:**
**Schwerk A**, Altschüler J, Roch M, Gossen M, Winter C, Berg J, Kurtz A, Akyüz L, Steiner B. Adipose-derived human MSC induce long-term neurogenic and anti-inflammatory effects and improve cognitive but not motor performance in a rat model of Parkinson's disease. *Regenerative Medicine*. 2015
**IF: 3.5 (2013)**
Contribution in detail: 80%. Performed the operations, executed behavioural experiments, executed and analysed histo- and cytostaining, performed stereological and non-stereological cell counting, executed multiplexed ELISA, performed statistical analyses and data interpretation, drafted and revised the manuscript, and partly did the correspondence to the reviewers.

Signature, date and stamp of the supervising University teacher

_____

Signature of the doctoral candidate

_____

## 4. Selected publications

**Study 1:** http://dx.doi.org/10.1007/s12015-014-9551-y

**Study 2:** http://dx.doi.org/10.1016/j.jcyt.2014.09.005

**Study 3:** http://dx.doi.org/10.2217/rme.15.17

## 5. Curriculum Vitae

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

## 6. List of publications

Verdejo-García A, Lubman DI, **Schwerk A**, Roffel K, Vilar-López R, Mackenzie T, Yücel M. Effect of craving induction on inhibitory control in opiate dependence. *Psychopharmacology* (Berl). 2012;219(2): 519-26.

**Schwerk A,** Alves FD, Pouwels PJ, van Amelsvoort T. Metabolic alterations associated with schizophrenia: a critical evaluation of proton magnetic resonance spectroscopy studies. *J Neurochem*. 2014;128(1):1-87.

**Schwerk A**, Altschüler J, Roch M, Gossen M, Winter C, Berg J, Kurtz A, Steiner B. Human adipose-derived mesenchymal stromal cells increase endogenous neurogenesis in the rat subventricular zone acutely after 6-hydroxydopamine lesioning. *Cytotherapy*. 2015;17(2):199-214.

Berg J, Roch M, Altschüler J, Winter C, **Schwerk A**, Kurtz A, Steiner B. Human adipose-Derived Mesenchymal Stem Cells Improve Motor Functions and are Neuroprotective in the 6-Hydroxydopamine-Rat Model for Parkinson's Disease when Cultured in Monolayer Cultures but Suppress Hippocampal Neurogenesis and Hippocampal Memory Function when Cultured in Spheroids. *Stem Cell Rev*. 2015; 11(1):133-49.

**Schwerk A**, Altschüler J, Roch M, Gossen M, Winter C, Berg J, Kurtz A, Akyüz L, Steiner B. Adipose-derived human MSC induce long-term neurogenic and anti-inflammatory effects and improve cognitive but not motor performance in a rat model of Parkinson's disease. *Regenerative Medicine*. 2015

## Contributions to Conferences

8th FENS Forum of Neuroscience, July 2012. Barcelona, Spain.
*Contribution*: Pericyte characterization of glial-derived neurotrophic factor expressing human mesenchymal stem cells from adipose tissue in the 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease. (poster)

Satellite event of FENS Forum "Regulation of adult neurogenesis: from epigenetics to behaviour". July 2012. Barcelona, Spain.
*Contribution*: Pericyte characterization of glial-derived neurotrophic factor expressing human mesenchymal stem cells from adipose tissue in the 6-hydroxydopamine (6-OHDA) rat model of Parkinson's disease. (poster)

The 41st Annual meeting of the Society for Neuroscience. November 2011. Washington, USA.
*Contribution:* Histological and clinical effects of glial-derived neurotrophic factor (GDNF) expressing human mesenchymal stem cells (MSC) from adipose tissue in the 6-OHDA rat model of Parkinson's disease. (poster)

The International Society of Psychoneuroendocrinology (ISPNE) meeting "Circadian Rythms". August 2011. Berlin, Germany.
*Contribution*: Histological and clinical effects of glial-derived neurotrophic factor (GDNF) expressing human mesenchymal stem cells (MSC) from adipose tissue in the 6-OHDA rat model of Parkinson's disease. (poster)

PhD Symposium "Stem Cells: Hopes, Fears and Reality", Berlin-Brandenburg School for Regenerative Therapies (BSRT). December 2010. Berlin, Germany.
*Contribution*: Histological and clinical effects of GDNF expressing human mesenchymal stem cells from adipose tissue in the 6-OHDA rat model of Parkinson's disease. (poster)

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