The role of β-catenin in the development of neural crest stem cells

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In metazoan organisms, β-catenin (β-cat) is a protein that plays a dual role as an important subunit in the cadherin-based cell-cell adhesion complex. In addition, b-cat acts as a central regulator of gene expression in the canonical Wnt-signaling pathway, which regulates decisions of the cell fate during development.
To distinguish the two roles of β-cat in development, we compared a conditional null mutant to a new mouse line (signaling mutant) with an altered β-cat allele from which a β-cat protein is produced that preserves adhesion, but lacks the ability to mediate Wnt-signaling.
We analyzed the development of the migrating neural crest stem cells (NCSC) in both mutants, and witnessed that Wnt signaling regulates lineage specific proliferation in NCSC. Furthermore, we concentrated on the trunk NCSC population expressing the basic helix-loop-helix factors (Neurogenin 2 and Neurogenin 1), which are necessary for neural differentiation, and give rise to the dorsal root ganglia (DRG). Our results show, that Neurogenin 2 is a target of β-cat mediated Wnt signaling, however the expression of Neurogenin1 is dependent only on the role of β-cat in cadherin mediated cellular adhesion. Furthermore, the subtype specificity of the sensory lineage of the DRG was analyzed in dependency of the effects evoked by the lack of β-cat mediated Wnt signaling.

Invitro differentiation of human bone marrow-derived stem cells towards retinogenic fate

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Purpose: Human stem cells promise to provide a well-characterized and reproducible source of replacement tissue for human clinical studies. An early potential application of this technology is the use of retinal pigment epithelium (RPE) for the treatment of retinal degenerative diseases. Hence this study sought to assess the *in vitro* differentiation capacity of human bone marrow-derived stem cells (BMSC) towards
retinogenic-like cells.

Methods: Samples of mobilized peripheral blood (mPB) and the bone marrow (BM) were collected from cancer patients and granulocyte colony-stimulating factor (G-CSF) mobilizations were used for the PB. Mononuclear cells (MNC) were isolated using Ficoll- Paque density gradient centrifugation, and were sorted by magnetic activated cell sorting (MACS) for specific BMSC populations. These cells were than cocultured on human RPE for 7 days. Cell morphology, expression of RPE-specific markers, stem cell, neural cell markers were examined by immunostaining. In addition, the expression profiles of specific BMSC populations were assessed by reverse transcription quantitative polymerase chain reaction (qRT-PCR) after FACS separation.

Results: Populations of PB $CD34^{+}CD38^{+}$ and $CD34^{+}CD38^{-}$ cells were isolated with 0.63 ± 0.42%, and 0.4 ± 0.27% of total BMC whereas, 3.38 ± 0.31% and 1.91 ± 2.24% of total BMC were in BM. After 7 days of co-culture the progenitor cells adopted an RPE-like elongated morphology and immunocytochemistry showed expression of RPE markers such as RPE65. The numbers of $CD34^{+}CD38^{+}$ cells were sorted by FACS after co-culture ranged from 0.73 ± 0.89% to 9.21 ± 1.12% of the input cell number of both PB and BM. In addition, RT-qPCR analysis of $CD34^{+}CD38^{+}$ cells showed up-regulation of stem cell markers (GATA2 and ß2M) down-regulation of RPE-specific (RPE65, BEST and MITF), and neural markers (NEST and ßIIIT). However, $CD34^{+}CD38^{-}$ cells revealed down-
regulation of stem cell and up-regulation of neural markers.

Conclusions: Our data demonstrated that human BMSC may differentiate towards retinogenic -like cell type *in vitro* and become a new type of donor cells for regenerative therapy in retinal degenerative diseases.

Therapy of perinatal brain damage by transplantation of human umbilical cord-derived mesenchymal stem cells in a rat model

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OBJECTIVE: Premature birth is a major cause of neonatal morbidity and mortality. Although improvements in perinatal and neonatal care have been made, it still remains a serious challenge. Enormous neurological problems such as cognitive, behavioral, attentional, or socialization deficits and major motor deficits categorized as cerebral palsy may follow. The aim of the study was to assess therapeutic effects of intracranial human mesenchymal stem cell (MSC) transplantation in a perinatal rat model of periventricular leucomalacia.
METHODS: The study was conducted in a sham controlled design. Intraperitoneal administration of lipopolysaccharide (Escherichia coli) followed by ligation of the left carotid artery and hypoxia (8% O2, 40min) was performed on anesthetized rats (postnatal day 4). Two therapeutic approaches were chosen. Human MSC derived from umbilical cords' Wharton's jelly (250`000 cells) were transplanted into the lateral ventricle using a stereotactic frame on postnatal day 6 or day 10. Some of the animals received erythropoietin (EPO) (1000 U/kg BW, i.p., 1 dose per day for 3 days) in combination with stem cell therapy or alone. The induced damage and the transplant were detected in brain sections and evaluated by histology and immunohistochemistry. Functional outcome was evaluated by footprint and walking pattern test.
RESULTS: Donor cells were detected in the brain post transplantation. Morphologic assessment of the induced brain damage indicates periventricular leucomalacia. Administration of stem cells alone and in combination with EPO reduced the extent of the damage. Functional test indicate a significant reduction of spastic paresis.
CONCLUSION: Transplantation of human placenta-derived MSC into the lateral ventricle of neonatal rats +/- EPO is possible. Morphologic changes in the recipients' brain indicate neuroprotective effects of the stem cell therapy. Further experiments will assess the window of opportunity of stem cell therapy in perinatal brain damage

Optimization of the human-Chorion derived Mesenchymal Stem Cells for Neuroregeneration to treat Pre- and Perinatal Diseases.

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Introduction: Mesenchymal stem cells (MSC) from the chorion membrane of the placenta can differentiate into neural lineages. MSC could be a source for cell transplantation as they are easily accessible, less immunogenic and ethically acceptable compared to the embryonic stem cells.

Objective: Our aim is to provide an optimized MSC graft for pre- and perinatal brain injury. It is done by altering the microenvironment of the MSC and by supplementation with a neurotrophic factor called hepatocyte growth factor (HGF), which could improve the migration, proliferation and differentiation capability of the cell graft.

Study Design: MSC isolated from chorion of term placenta were subjected to 0.1 % gelatin-coated culture flasks/serum replacement (G^+S^-), or uncoated culture flasks/fetal calf serum (G^-S^+). MSC are characterized by FACS and cell proliferation was assessed by MTS assay. Various growth factors, chemokines and cytokines released from the MSC were quantified using Bio-plex pro Assay. Expression of neural progenitor cell markers was analyzed by FACS and qRT- PCR. Differentiation of MSC was carried out according to Zhang et al. Differentiation 2010, 79:15 and Fu et al. Acta Neurobiol Exp 2007, 67:367.

Results: Expression of MSC markers was independent of culture conditions. G^+S^- conditions resulted in a higher proliferation rate and higher expression of neural progenitor markers as compared to G^-S^+ conditions. Bioplex results confirm that G^+S^- cells produces more essential growth factors, chemokines and cytokines compared to G^-S^+ cells. MSC were differentiated into neural stem cells and supplemented with HGF, which up-regulated the migratory-chemokine receptors such as cMET (HGF receptor) and CXCR4 (SDF-1 receptor).

Conclusion: The combination of gelatin-coating, serum replacement and HGF supplementation could possibly result in a cell population optimized for neural differentiation and could improve the efficacy in cell therapies for pre- and perinatal brain injury.

The role of Ski in neurogenesis

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In embryonic development neural stem cells form the neuroepithelium by self-renewal and build a pool of progenitors that start differentiating to neurons to form the six-layered cortex. We study the role of Ski (Sloan-Kettering viral oncogene homologue) during cortical development. Ski is a transcriptional regulator that interacts with DNA-binding protein complexes and promotes cell proliferation and specification of cortical neurons. In vivo, Ski is expressed in apical neuronal progenitors in the ventricular zone and in cortical neurons in the dorsal telencephalon. The analysis of Ski-deficient cortices revealed a change in cell cycle characteristics of progenitor cells and a switch in the identity of callosal neurons.

To study the function of Ski in cortical progenitor cells, we use a cell culture system that allows us to differentiate WT and Ski KO embryonic stem cells to radial glia progenitor cells and postmitotic neurons. We find that Ski is highly expressed in Sox2 and Nestin-positive progenitor cells, and that loss of Ski affects their proliferation and differentiation behavior. Ski-deficient neural progenitors proliferate less and differentiate prematurely into neurons. Mutant neurons not only show morphological changes but also need particular culturing conditions for survival and further maturation. My aim is to identify Ski-interacting partners and signaling pathways that are regulated by Ski and control the progression from proliferating neural progenitor cells to differentiated neurons. Ski is known to repress TGFbeta signalling by inhibiting Smad function. I will present new data on the role of Ski in neurogenesis and Ski with TGFbeta signalling in neural development.

Adult neurogenesis in *Drosophila*

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Appropriate brain development is crucial to precisely coordinate the plethora of complex functions in animals, concerning no just internal homeostatic processes but also social and environmental interactions, like communication, learning and memory. This is specially recognized in neuro-degenerative diseases, where some times the brain suffers irreversible damage. Therefore, it is of great value to have a suitable model organism to study adult brain regeneration and plasticity. With its amenability to handle, highly conserved genome and impressive genetic toolbox, *Drosophila melanogaster* is a suitable model to study this processes. Nevertheless, nowadays adult neurogenesis has been recognized in different organisms but not in the fruit fly, mainly because generation of new neurons in adult brains has not been identified and the presence of neural progenitors has been thought to be absent.
By improvement of a previously reported system, we generated “Perma-twin”, a mitotic recombination-based lineage labeling method, and have identified generation of new neurons in the adult fly brain during adulthood, that is surprisingly increased in response to induced acute brain damage, that clearly shows an adaptive regenerative plasticity. In addition, we have also identified in adult brains a new cell type expressing Deadpan, a larval neural stem cell marker, with proliferative potential. Finally, we identified a factor which expression is sufficient to trigger proliferation in such adult neural stem-like cells. Our results reveal unexpected plasticity in the adult *Drosophila* brain, and describe a unique model for the genetic analysis of adult neurogenesis, regeneration upon acute brain damage and adult neural plasticity. Basic knowledge gained with this model organism can be easily transferred to higher organisms, boosting the research in neural stem cells biology.

Human Wharton's jelly-derived mesenchymal stem cells express neurotrophic factors in vitro

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OBJECTIVE:
Perinatal brain damage is a major neurological problem in surviving premature infants. Recent transplantation experiments in various animal models suggest a neuro-regenerative potential of multipotent mesenchymal stem cells (MSC). The curative effect of MSC might be due to their production of neurotrophic factors. The umbilical cord connective tissue (Wharton's jelly) represents a promising source of MSC. Thus, the aim of the study is to assess the expression and release of neurotrophic factors by human Wharton's jelly-derived MSC and induced neural progenitor cells in vitro.
STUDY DESIGN:
MSC from Wharton's jelly of term and pre-term (gestational age < 37 weeks) pregnancies were evaluated. Adaptations of previously published multistep protocols (Portmann-Lanz et al, AJOG 2010; Fu et al, Acta Neurobiol Exp 2007; Zhang et al, Differentiation 2010) were used to produce neural progenitors (neurospheres). The transcription of neurotrophic factors was assessed by real-time PCR. The release of neural growth factors into the cell culture medium was measured by a membrane-based cytokine antibody array.
RESULTS:
At passage five MSC from term and preterm pregnancies were expressing key neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NTF3) and glial cell-derived neurotrophic factor (GDNF), and the cytokine interleukin (IL)-6, at the mRNA level. BDNF and IL-6 were detected in the cell culture supernatant after 48h of cultivation. The transcription of BDNF and NTF3 were significantly reduced in neurospheres relative to MSC, independent of gestational age. However, the gene expression of GDNF was up-regulated in neurospheres compared to the non-induced MSC derived from term pregnancies.
CONCLUSIONS:
MSC derived from Wharton's jelly of term and preterm pregnancies, and the induced neural progenitor cells produce neurotrophic factors in vitro. The role of the released factors in neurogenesis and neuro-regeneration is currently analyzed in co-culture experiments with neural stem cells.
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Derivation of traceable photoreceptors from embryonic stem cells

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Retinal degenerative diseases resulting in the loss of photoreceptors are a major cause of blindness. Recently, different groups validated the possibility to reactivate dormant or establish new retinal circuits of degenerating retinas focusing on approaches aimed to create/transplant new photoreceptor (retinal prosthesis, gene therapy, cell replacement therapy). Photoreceptor replacement therapy may be feasible since transplanted photoreceptors, collected directly from the developing or the adult retina, have been shown to restore some visual function in degenerating retinas. Because the developing retina is not a suitable source of renewable photoreceptors, we focused on embryonic stem cells (ESCs) for their capacity to generate retinal progenitors and photoreceptor cells in vitro. In this study we examined a recently published protocol for its potential to give rise to 3D-optic cups from a three-dimensional culture of mouse ESC aggregates (Eiraku et al., 2011). RT-PCR, immunohistochemistry, activation of a retinal progenitor-specific reporter, the *Rx-GFP* transgene, were used to assess the rate of optic cup formation. We observed that during the *in vitro* self-formation, retinas do not differentiate in a synchronized fashion and that the differentiation process depends at least on ESC strain, batch of matrigel, interaction among different aggregates, number of optic vesicles grown inside the same aggregate. All these variables render the timing of photoreceptor generation quite heterogenic. In order to characterize newly generated photoreceptors, we derived a new transgenic ESC line in which the reporter gene, the *Crx-GFP* transgene, is expressed in post mitotic photoreceptor precursors. Using the 3D-optic cup induction protocol, GFP-positive photoreceptors appeared in a punctuated pattern in the optic-cup like structures around day 12 and then starting from day 18, forming a fluorescent layer of aligned GFP-positive cells. Such structures are now under characterization (RT-PCR, immunohistochemistry, cell sorting) to reveal the developmental stage of *in vitro*-differentiated photoreceptors at different time points and to evaluate which stage is the most appropriate for cell transplantation and for other biological studies.

Ezh2 regulates neural stem cell fates in the developing mouse midbrain

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Size and growth of the developing brain is determined by the choice of multipotent neural stem cells (NSC) between self-renewal and differentiation. The polycomb group protein Ezh2, enhancer of Zeste homolog 2, is the catalytic subunit of polycomb repressive complex 2 (PCR2) and is primarily responsible for trimethylation of histone H3K27 (H3K27me3). This epigenetic mark contributes to repression of many genes, which are pivotal for neural development. Here we show that Ezh2 is essential for the regulation of neural progenitor (NP) fate decision in the developing mouse midbrain. Wnt1-Cre-mediated ablation of Ezh2 results in a shortened caudal midbrain most likely caused by impaired NP proliferation from embryonic day (E) 12.5 onwards. In addition mutant NP exit the cell cycle precociously resulting in increased neural differentiation. Mutant midbrains also show a downregulation of pro-proliferative Wnt/β-catenin signaling. To further dissect the role of Ezh2 in midbrain development we performed whole-genome transcriptome analysis of mutant and control midbrains. This approach now allows us to identify direct repression targets of Ezh2 and their role in the regulation of Wnt/β-catenin signaling in the midbrain.

What is the Benefit of Grafting Bone Marrow Derived Stem Cells (BMSC) into the Hippocampus after Bacterial Meningitis?

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Background
Pneumococcal meningitis (PM) causes apoptosis in the hippocampus, a region critical for memory acquisition and harboring a stem cell niche. We hypothesize that the injury to the stem cell niche may impede endogenous repair mechanisms which in turn cause persisting neurofunctional deficits in patients surviving PM. We have recently demonstrated that transplanted fetal neuronal stem/precursor cells in rats after PM migrated into the damaged hippocampal region and differentiated into an immature neuronal phenotype. Transplantation of bone marrow derived stem cells (BMSC) may either replace cells lost during PM or modulate beneficially the local immunologic environment by production of trophic factors.
Aim
To evaluate the fate and the effect of BMSC grafted into the hilus region of the hippocampus after experimental bacterial meningitis *in vitro* and *in vivo*.
Methods
Isolation of BMSC: BMSC were isolated by flushing femur and tibias of transgenic rats expressing green fluorescent protein (GFP). They were cultivated in culture medium (DMEM/F-12, FCS, antibiotic-antimycotics) for 2-5 passages in 75cm2 cell culture flasks to select for mesenchymal stem/stromal cells (MSC) by plastic adherence.
Organotypic slice cultures: MSCs were grafted into the dentate gyrus hilus of organotypic hippocampal slice cultures injured by challenge with live *Streptococcus pneumoniae*. Migration and differentiation of grafted cells were assessed by immunohistochemistry.
In vivo model: PM was induced in infant rats by injection of live *S. pneumoniae;* antibiotic treatment (ceftriaxone) started 18h later. 0.1-1x105 MSCs in 10 µl PBS were injected intracisternally between 72 h and 1 week after cured PM. One week after transplantation, rats were sacrificed, perfused with formaldehyde and coronary brain slices were collected to screen for survival and migration of transplanted cells.
Results
When grafted in hippocampal slices, injured by pneumococcal challenge, BMSCs showed no migratory activity but remained stationary at the site of transplantation in the hilus region of the hippocampal dentate gyrus and formed undifferentiated cell clusters. Preliminary results from *in vivo* studies showed only sporadic presence of surviving BMSC at the injection site.
Significance/Outlook
Transplanted BMSC are not appropriate for therapeutic approaches aimed at replacing injured stem cells in the hippocampal dentate gyrus. Further *in vivo* studies will evaluate a neuroprotective effect of BMSC in the acute disease phase and a neuroregenerative effect in long-term disease outcome. Primary endpoints assessed will be histomorphometric analysis including evaluation of neurogenesis with BrdU studies and neurofunctional outcome. Identity of cultured BMSCs will be confirmed by FACS analysis. The transplantation of MSCs may hold promise for regenerative therapies aimed at repair of brain damage after PM.

Evaluation of the Mood-Stabilizer Lithium on acute brain injury in Experimental Pneumococcal Meningitis

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Background
Pneumococcal Meningitis (PM) causes high mortality and morbidity and leads to persisting sequelae in more than 50% of affected children. The survivors show deficits in academic, executive and intellectual performance, which persist into adulthood. The histomorphologic correlates of these deficits include injury to the brain characterized by apoptosis in the dentate gyrus (DG) of the hippocampus and necrosis in the cerebral cortex. We hypothesize that the observed injury to the hippocampal stem cell niche may impede endogenous repair mechanisms, which in turn, causes persisting neurofunctional deficits in patients surviving PM. Protecting the stem cell niche during acute disease and supporting regeneration via neurogenesis after PM are potential targets for therapy.
In addition to the well-documented mood-stabilizing effects of lithium in patients suffering from manic-depressive illness, several studies have shown neuroprotective and neuroproliferative effect in paradigms of acute and chronic neurodegenerative diseases.
Aim
To assess the effect of systemic administration of lithium on brain injury in an infant rat model of PM.
Methods
5 days prior to the induction of PM, animals were randomly assigned to lithium treatment (63 mg/kg body weight, s.c., daily) or vehicle (saline) as control group. PM was induced by intracisternal injection of live Streptococcus pneumoniae (serotype 3) in 11 days old Wistar rats. 18 hours post infectionem, when animals developed disease symptoms, antibiotic treatment with ceftriaxone (100 mg/kg body weigt, i.p. bid) was initiated. PM was confirmed by quantitative culture of cerebrospinal fluid (CSF) samples on blood agar plates. Animals were sacrificed 42 hours after infection, blood collected and brain removed for histomorphometric analysis (quantification of apoptotic cells in DG and evaluation of damaged cortex volume).
Serum concentrations of lithium (target range 0.4 – 1.4 mM) were determined by capillary electrophoresis.
Results
A significant reduction ($p<0.05$, Mann Whitney U Test) of the number of apoptotic cells in the hippocampal DG was observed in animals with confirmed lithium serum concentrations between 0.4 mM and 1.4 mM (n=9, saline n=25). No differences in survival proportions, weight differences, clinical scores during acute PM and volume of cortical damage was observed between saline and lithium treated animals.
Summary
In experimental PM lithium acts neuroprotective by attenuation of apoptotic brain injury in the stem cell niche of the hippocampus.

The role of Wnt signaling in regulating radial migration and positioning of late-generated pyramidal neurons

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Cortical layers are generated by precisely coordinated cell migration events and the mammalian cortex is particularly susceptible to disorders of migration. Understanding the mechanisms and regulation of migratory events is therefore crucial to understand how alterations in this process might contribute to neurodevelopmental disorders. It has long been known that the Wnt signaling pathway plays a central role in many aspects of corticogenesis, including neural progenitor proliferation and neuronal differentiation, but its direct role in regulating cell migration and positioning remained unknown. We have developed a set of molecular tools and introduced them into proliferative cells in the in vivo developing cortex using in utero, intraventricular injections and electroporation at E18. We found that the canonical Wnt/β-catenin signaling is active in radially migrating cells. Moreover, the inspection of migrating cells at different developmental time-points revealed remarkable effects of gain-of-function and loss-of-function strategies, including alteration in cell polarity, cell locomotion and positioning. Together these preliminary findings provide the first demonstration that Wnt signaling is required for the radially directed migration of late-generated pyramidal precursors.

Intraventricular infusion of endothelial progenitor cell conditioned medium promotes endogenous neurogenesis

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Background: Insults of the brain has been shown to promote endogenous neurogenesis in the subventricular zone. Its extend, however, is considered to be insufficient for functional brain repair. In line with this notion, there is increasing evidence for a regenerative potential of soluble factors released from stem and progenitor cells. We have previously shown that Endothelial progenitor cell Conditioned Medium (EPC-CM) promoted survival of cultured neuronal cells. In the present study we investigated the effects of EPC-CM on the neuronal stem cell niche in the subventricular zone of adult rats. Methods: EPC were isolated from peripheral blood of healthy human donors by gradient centrifugation. Cells were cultured in hypoxic conditions (1.5% O2) for 1 day to enhance the secretion of growth factors. Adult rats were anesthetized (Ketamin and Xylazin, i.p.). After a cranial midline incision EPC-CM was infused by means of a mini osmotic pumps implanted into the right lateral ventricle. Basal cell culture medium was used as control. The infusion rate was 0.5ul/h for 3 days. Animals were injected daily with the proliferation marker BrdU intraperitoneally. At the end of the experimental period the rats were perfusion fixed using 4% PFA and the brains sectioned on a cryostat. Brain slices were immunostained for BrdU and markers of progenitor cells and stem cells.
Results: Intraventricular infusion of EPC-CM was observed to significantly increase the number of subventricular BrdU positive cells as compared to controls. Similarly, Ki-67, Vimentin and GFAP positive cell numbers were significantly higher in the EPCCM treated group as compared to controls. Importantly, we could demonstrate that the number of doublecortin expressing neuronal progenitor cells were significantly augmented after EPC-CM administration as well as stem cell markers Oct-4 and Sox-2.
Conclusions: In sum, our findings demonstrate that EPC-CM administration resulted in enhanced cell proliferation and promoted endogenous neurogenesis in the subventricular zone. These observations indicate that EPC-CM may be offering a novel therapeutical strategy to induce neuroregeneration in the disease brain

From Blood to Brain: Contribution of Endogenous Bone Marrow-Derived Mesenchymal Stem Cells to Brain Repair After Stroke

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Recent studies have highlighted the possibility that endogenous bone marrow derived stem cells (BM-MSCs) have the potential to give rise to cells of the neural lineage in the mammalian brain. However, it is not known whether this type of endogenous repair might contribute to production of new neurons after ischemic stroke.

In the present study, we therefore investigated the neurogenic potential of BM-MSCs in chimeric mice that underwent whole body irradiation followed by BM reconstitution with green fluorescent protein (GFP) expressing transgenic BM-MSCs. Recruitment and differentiation of GFP-expressing cells was addressed in uninjured controls and in two different murine stroke models, the permanent distal middle cerebral artery occlusion (dMCAO) model and the hypoxia-ischemia (HI) model, which allows reperfusion of the ischemic parenchyma after induction of the insult.

Mice were sacrified at 4 weeks or 3 months after stroke, respectively, and the brains were processed for immunohistochemistry. Co-localization studies were carried out with GFP and the neuronal markers DCX, NeuN and Tuj1, the astrocyte marker GFAP, the oligodendrocyte progenitor marker NG2, the macrophage/microglia marker Iba1 and the endothelial cell marker vWF.

In both the dMCAO and the HI model, we found engraftment of GFP-expressing BM-MSCs, particularly in the stroke borderzone, at 4 weeks and 3 months after the insult. GFP-positive BM-MSCs-derived cells did not co-localize with DCX, NeuN, Tuj1, or NG2. However, abundant co-localization was present with Iba1- and vWF-immunoreactive cells, and sparsely, GFAP-positive astrocytes also co-expressed GFP. The number of GFP-positive cells in the brain as well as the co-localization with Iba1 and vWF were significantly higher in mice after dMCAO or HI when compared to non-stroked controls.

Therefore, our data suggest that endogenous BM-MSCs in the systemic circulation are recruited to the post-ischemic murine brain, but do not contribute to the generation of new neurons. On the other hand, our findings further support the importance of microglia and endothelial progenitors derived from BM-MSCs for the inflammatory response and angiogenesis after ischemic stroke.

Effects of GHB and baclofen on sleep and motor function in healthy rats and rats with focal cerebral ischemia

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Objectives: Ischemic stroke remains one of the leading causes of death worldwide. However, there is still no available effective treatment for stroke patients. Promoting neuroplasticity during recovery may represent an alternative strategy in development of new stroke therapy. There is strong evidence that sleep is important in facilitation of neuroplasticity. Sleep promoting drugs, gamma-hydroxybutyrate (GHB) and baclofen (Bac), showed neuroprotective effects when given within 24 hours after ischemia. Our goals were: 1) to check the effects of GHB and Bac on sleep in healthy rats and to determine the optimal drug dose, 2) to evaluate sensorimotor function recovery after delayed repeated treatment with Bac in a rat model of focal cerebral ischemia.

Methods: 1) Adult male rats (n=26) were implanted with EEG/EMG electrodes and assigned to the GHB (150 or 300 mg/kg), Bac (10 or 20 mg/kg) or saline treatment group. Injections were performed 1h after light onset and offset to evaluate time of day effect of the drugs. Every rat received two injections. 2) 24h after initiation of focal cerebral ischemia (permanent occlusion of the distal branches of middle cerebral artery, MCAo) rats were treated with Bac (10 mg/kg) or saline. Then injections were given twice daily during 10 consecutive days. Ischemia/Bac (n=14), ischemia/saline (n=14) and sham/Bac (n=8) groups were designed. Sensorimotor function was evaluated by single pellet reaching test (SPR) every week in the course of 1.5 month after MCAo.

Results: 1) GHB and Bac induced atypical animal behavior and altered EEG pattern . The drugs effect lasted up to 413 min. Amount of vigilance states was evaluated after the end of the drug effect. Bac treatment resulted in the increase in NREM sleep by 16 min in the light phase ($p<0.05$, paired t-test) and by 91 min in the dark phase ($p<0.001$). REM sleep was enhanced by 12 min in the dark ($p<0.05$). Duration and frequency of NREM sleep episodes depended on the timing of Bac administration. Thus, during the light phase Bac increased the duration of NREM sleep episodes ($p<0.01$), but reduced their frequency ($p<0.05$), while during the dark phase it increased the episode frequency ($p<0.0001$). In addition, Bac administered during the dark phase reduced sleep fragmentation ($p<0.001$, paired t-test). GHB had no major effect on the amount of vigilance states.

2) SPR performance dropped to 0 immediately after MCAo in both saline and Bac treated rats. Thereafter, both groups showed a slow recovery of function. No significant difference was observed between ischemia/Bac and sham/Bac groups 33 days after surgery. In contrast, ischemia/saline rats never reached the performance level of sham operated animals. Moreover, ischemic rats treated with Bac performed significantly better than saline treated rats ($p=0.01$, Tukey-Kramer).

Conclusion: Our results demonstrate that: 1) GHB and Bac induced sub-anesthetic state distinct from physiological sleep, confirming previously published mouse data. In contrast to GHB, Bac treatment increased the amount of sleep after the end of drug effect. 2) Delayed repeated Bac treatment might benefit motor function recovery after ischemic stroke.

Keywords: ischemic stroke, sleep, motor function, GHB, baclofen

Major keyword: ischemic stroke (?) or sleep (?)

A new behavioral analysis in rats to understand motor fluctuations in parkinsonian patients treated with L-DOPA

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Locomotor disorders like bradykinesia (slowness of movement) or hesitation of gait initiation are a hallmark of Parkinson's disease (PD). In the treatment of PD, Levodopa (L-DOPA) remains the most effective drug. However the majority of parkinsonian patients under L-DOPA therapy develops disabling motor complications like motor fluctuations, characterized by on/off phenomena, and abnormal involuntary movements, called dyskinesia.
The purpose of our study is to establish a behavioral animal model which is close to the locomotor activity performance of parkinsonian patients both during and in the absence of L-DOPA therapy.
Parkinsonian rats with 6-hydroxydopamine (6-OHDA) lesions were treated with chronic intraperitoneal injections of L-DOPA (8 mg/kg) once a day for 21 consecutive days; another group of lesioned rats received chronic injection of NaCl (vehicle) and a third group of naive animals received the standard L-DOPA chronic treatment. The motor activity of all groups was valuated using behavioral analysis software (Ethovision) at 19nd day of L-DOPA and NaCl treatment (ON period) and at 22nd day without L-DOPA and vehicle administration (OFF period), respectively.
Results: Preliminary observations about motor performance in parkinsonian rats demonstrated an higher locomotor activity, expressed as "total distance moved" and "mean velocity", in animals during the L-DOPA ON period than during the L-DOPA OFF period. Furthermore, Levodopa-induced dyskinesia were seen only in the ON-state, similar to what is seen in parkinsonian patients under L-DOPA therapy. On the contrary, no difference in motor activity was found in the parkinsonian rats treated with NaCl during ON/OFF period and naive groups during L-DOPA ON/OFF treatment, respectively.
We conclude that our animal model's behavior is close to motor performances in human suffering from ON/OFF motor fluctuations. This experimental model is better suited for pre-clinical study in order to test the use of new pharmacological drugs, able to reduce the motor complications induced by L-DOPA treatment.

Inner ear stem cells for tissue regeneration of the auditory organ

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Hearing impairment is the most frequent human sensory deficit and is mainly caused by the irreversible loss of neurosensory cells in the cochlea.
Previous studies have shown the presence of stem/progenitor cells within the mammalian auditory epithelium, vestibular organs and spiral ganglia, however the regenerative capacity of these organs is extremely poor, in contrast to birds and amphibians.
Stem/progenitor cells can be isolated through a sphere-forming assay in vitro or through prospective isolation using FACS and can give rise *in vitro* to the differentiated cell types of the tissue of origin. We are currently investigating a number of approaches to efficiently and reliably isolate and expand these cells from rodent as well as human adult and fetal tissues.
Stem cell culture *in vitro* provides in fact a useful tool for drug screening to analyze hair cell regeneration and discover novel neurotrophic factors to increase auditory neurons survival.
One project of the laboratory applies stem cell technology for regenerating the auditory nerve in order to improve current cochlear implant (CI) technology. A CI restores hearing in deaf patients through direct electrical stimulation of auditory neurons in the cochlea. Despite its success, current CI technology is limited through a low resolution of the auditory signal, which is a consequence of overlapping electrical fields and anatomically due to the distance of the stimulating electrode in the scala tympani to the auditory neurons located in the spiral ganglion. A gapless interface between neurons and the electrode would be an elegant solution to this problem, however, has not been attempted previously. An EU-FP7 funded project (NANOCI), of which we are coordinators, has been launched recently with the aim of developing a novel electrode type, specifically modified in order to release neurotrophic and chemoattracting factors and that will induce neuronal sprouting, guided migration and stable locking of the nerve endings on the electrode surface.
Another project addresses stem cell based regeneration of auditory neurons in the context of bacterial meningitis. In collaboration with the group of Prof. S. Leib, (Neuroinfectiology University of Bern), transplantation of neuronal progenitors into postmeningitic deaf rats is performed. Preliminary data indicate that the surgical approach is feasible and that transplanted cells survive and morphologically integrate into the modiolus of the inner ear