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Brain poster session: neurogenesis

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71. Live imaging of stroke induced neurogenesis in the mouse brain

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Background and aims: Neurogenesis is upregulated following stroke, and may contribute to brain repair. Neurogenesis is typically studied invasively. Recently, transgenic mice were generated that express either Discoma sp reef coral red fluorescent protein (DsRed) (Couillard-Després *et al. Eur J Neurosci* 2006;24: 1535–45), or the bioluminescent enzyme luciferase (LUC) (Couillard-Després *et al. Mol Imaging* 2008; 7:28–34), under the control of the migrating neuroblast promoter doublecortin (DCX). Therefore, the aims of this project were to employ noninvasive multimodal imaging to observe the timecourse and expression patterns of the neurogenic response following focal ischemia, *in vivo*.

Methods: Male CD1 DCX-LUC mice (37 to 44 *g*) ($n = 4$) and C57/BL6 DCX-DsRed mice (29 to 35 *g*) ($n = 3$) received different durations (30, 20, or 10 mins) of transient middle cerebral artery occlusion (MCAO); one DsRed mouse received a sham procedure. Optical imaging was performed to detect fluorescence, or bioluminescence (using intraperitoneal administration of 150 mg/kg of luciferin), at 23 days prior to and 12 days post MCAO. Anatomical magnetic resonance imaging (MRI) scans (spin echo T_2-weighted, and 3D fast low angle shot (FLASH) images) were acquired 4 days prior to and 7 days post-MCAO at 11.7 T. Two DsRed mice (including the sham) were sacrificed at 7 days post-MCAO and the other animals at 12 days. Brain tissue was processed for fluorescent immunohistochemistry for the imaging reporters in combination with DCX and GFAP.

Results: Ischemia durations of 30 mins produced extensive lesions and mortality, as observed from T_2 maps, whereas smaller subcortical insults were produced using 10 to 20 mins of occlusion. No fluorescent signal was detected from the C57/BL6 DCX-DsRed animals *in vivo*, but photons produced from the oxidation of luciferin in the CD1 DCX-LUC mice were observed and counted. Emission was highest in the head in a pattern similar to the pathway of the rostral migratory stream in the brain (Figure 1C and D). Following MCAO the bioluminescent signal increased and appeared to shift laterally towards the injured hemisphere (Figure 1E and F). Improved localization will be possible using co-registration of the optical images to the FLASH 3D MRI data set (Figure 1A and B).

Conclusions: These preliminary results indicate that monitoring the neurogenic response *in vivo* follow-

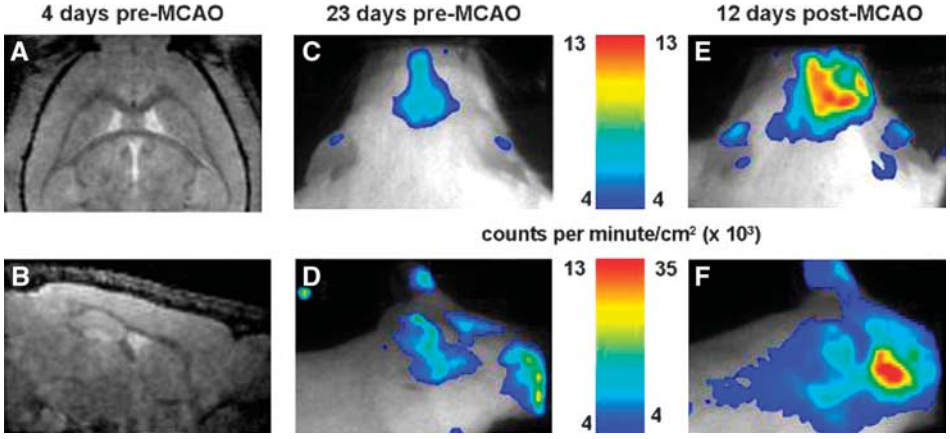

Figure 1 (**A** and **B**): Horizontal and sagital FLASH images from a CD1 DCX-LUC animal at 4-days prior to mcao, (**C** and **D**): Corresponding bioluminescent images (counts/minute per cm^2) from the same animal 23 days prior to and 12 days post MCAO (**E** and **F**). Note the increase in bioluminescence after MCAO, and shift towards the ischemic hemisphere.

ing stroke is possible. Ongoing work is being performed to characterize the location, intensity, and timecourse of this response using a multimodal imaging protocol combined with histological validation.

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235. Brain plasticity and anti-depressant effects are versatile potential of alpha-linolenic acid to promote stroke recovery

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Objectives: Prevention and treatment of stroke, a major public Health concern in Western countries, are major challenges in modern medicine. Besides mortality and devastating neurovascular unit damages, post-stroke depression (PSD) is a frequent psychiatric complication (prevalence of 30%). PSD increases mortality rates and worsen functional outcomes. We have previously demonstrated that alpha-linolenic acid (ALA), a polyunsaturated fatty acid reduces the ischemic damage by limiting glutamate-mediated neuronal death. We aimed to create a global strategy based on three sequential injections of ALA for stimulating brain responses and decreasing the post-stroke psychiatric complications to achieve the best functional recovery.

Methods:

(1) The protective effects of three sequential injections of ALA as pre- or post-treatment against stroke were determined using the MCAO model in mice.
(2) The effect of single or repeated ALA-injection treatment on neurogenesis was evaluated studying BrdU incorporation in the hippocampal Dentate Gyrus and by immunohistochemistry with fluorescent double-labeling.
(3) Neuronal plasticity known to contribute to brain repair was evaluated measuring the expression of key proteins involved in synaptic functions, synaptophysin-1, VAMP-2, and SNAP-25 as well as proteins supporting glutamatergic neurotransmission, V-GLUT1 and V-GLU2, by Western blot.
(4) To investigate the functional-anatomical relationship between ALA and neurogenesis/synaptogenesis, we studied BDNF expression by RT-PCR, Western blot and ELISA.
(5) Neuroprotection, neurogenesis and synaptogenesis were also tested *in vitro* on neural stem cells and hippocampal cultures.
(6) ALA treatment was tested in the Porsolt Forced Swim Test and Tail Suspension Test, that are commonly accepted to predict antidepressant efficiency of drugs.

Results: Three sequential injections of ALA enhanced protection. As a pre-treatment, it reduced by approximately 30% the post-ischemic infarct volume 24 h post-MCAO. As post-treatment, it augmented neuronal survival rates by three-fold 10 days following ischemia. By themselves the three sequential injections of ALA increased neurogenesis 3 days after the end of the treatment. BrdU-positive cells increased by 1.5 in the ALA-injected mice as well as the number of neuronal progenitor cells Brdu-DCX positive by 50%. In the cortex, ALA treatment induced a parallel increase of synaptophysin-1 and VAMP-2, two vesicle-associated synaptic proteins and SNAP-25, their membrane-associated synaptic protein. It also increased the vesicular glutamate transporters V-GLUT-1 and -2 (key factors of the glutamatergic neurotransmission efficacy). These ALA effects were correlated with an *in vivo* increase in BDNF protein levels, which was confirmed *in vitro* on neural stem cells and hippocampal cultures. Because BDNF has antidepressant activity, we tested whether chronic ALA treatment could produce antidepressant-like behavior. ALA-injected mice had significantly reduced measures of depressive-like behavior compared to vehicle-treated animals, suggesting another aspect of ALA treatment that could boost functional stroke recovery by reducing psychiatric complication.

Conclusion: The present work demonstrates that treatment of experimental stroke with repeated ALA-injections improves protection. Beside to acute vasodilation and excitotoxicity limitation ALA displays versatile potential like enhancement of neurogenesis, synaptogenesis and neurotransmitter transmission. From a clinical point of view, this 'multi-target' effect of chronic ALA-treatment may represent a novel approach to stroke and subsequent PSD therapies.

376. Neurogenesis of motor neurons in the spinal cord after transient ischemia

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Objectives: Spinal cord injury after a successful operation on the thoracic aorta is a disastrous and unpredictable complication in humane beings. In an attempt to prevent this complication, various methods of spinal cord protection have been suggested, Because motor neurons in spinal cords have been thought not to replace after cell deaths. It has already shown that self-renewing stem cells do exist in discrete brain regions, including the dentate gyrus (DG) of the hippocampus and subependymal layer close to the subventricular zone (SVZ) of lateral ventricles. Recent studies have shown that adult neurogenesis is increased by an exogeneous neurotrophic factor supplement, seizures kindling an enriched environment and transient global ischemia, and is decreased by stress, excitatory amino acids, and adrenal steroids. On the other hand, motor neurons in anterior horns of spinal cord after transient ischemia has been thought to die with apoptotic change,[1] and do not replaced after cell death. In the spinal cord, neurogenesis cannot be observed under physiologic condition. However, a different situation occurs after pathological spinal cord injury.[2]

Because ischemic stroke often causes loss of the neural functions because of neural cell death, neurogenesis after ischemia should be important for compensation for and recovery of those functions. The stage of neurogenesis in the DG can be divided into three steps; (1) proliferation, (2) migration, (3) differantiation.[3]

In the present study, we evaluate the three steps of neurogenesis in spinal cord after transient ischemia.
Materials and methods: Male domesticated white rabbits (Japan) were subjected to reproducible models for spinal cord ischemia. To evaluate the three steps of neurogenesis after ischemia, we used bromodeoxiuridine (BrdU), highly polysialylated neural cell adhesion molecule (PSA-NCAM), and neural molecular antigen (NeuN), and glial fibrillary acidic protein (GFAP), as markers for proliferation, migration, and differentiation, respectively.
Results: BrdU-labelled cells and PSA-NCAM-positive cells increased in the anterior horns of spinal cord at four days after ischemia. BrdU-labelled cells with PSA-NCAM expression were first detected in the spinal cord after 4 days of transient ischemia. A few of PSA-NCAM labeled cells with NeuN was detected after 7 days, however no PSA-NCAM-labelled with GFAP was detected.
Conclusions: Our results indicate that ischemic stress could stimulate the ability of neurogenesis even in spinal cord. Our findings will be important in developing therapeutic intervention to enhance endogenious neurogenesis after spinal cord ischemia.

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381. Changes in cell death, gliosis and cell proliferation/differentiation in the amygdala following myocardial infarction

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Background and aims: Myocardial infarction (MI), also known as a heart attack, occurs when the blood supply to the heart is interrupted and finally this cause damage to myocardium. Recent studies

showed that depression is frequently observed following MI, suggesting a link between heart disease and brain function. It has been reported that the neuronal death are occurred in the amygdala 72 h after 40 mins coronary artery occlusion. The amygdala is a central component of the limbic system and plays a crucial role in behavioral responses to emotional stress.in recent study, new neurons are produced in the amygdala, piriform cortex, and adjoining inferior temporal cortex in adult primates. Although some researchers have demonstrated the neuronal death in the amygdala after MI, no studies have been reported on cell proliferation/differentiation and gliosis in the amygdala after MI in rats.
Methods: Induction of MI, Cresyl violet staining, Fluoro-Jade B (F-J B) histofluorescence staining and Immunohistochemistry for GFAP, Iba-1, Ki67 and DCX.
Purpose: In this study, we examined cell proliferation and neuroblast differentiation in the amygdala and morphological changes in astrocytes and microglia 2 weeks after MI, when behavioral signs compatible with depression are detected after cardiovascular events.
Results: In MI-operated group, cresyl violet positive neurons had condensed cytoplasm and Fluoro-Jade B-positive cells were detected. The cytoplasm of glial fibrillary acidic protein (GFAP) immunoreactive astrocytes and ionized calcium-binding adapter molecule 1 (Iba-1) immunoreactive microglia were hypertrophied and the processes in astrocytes and microglia were highly ramified and retracted in the amygdala. The number of Ki67 positive cells was significantly increased by 815% in MI-operated group compared to that in the sham-operated group. In addition, DCX immunoreactive neuroblast were abundant in the amygdala of MI-operated group.
Conclusions: The neuronal death, reactive gliosis and cell proliferation is promiment 2 weeks after MI. However, the neuronal differentiation is not detected this region. These results suggest that MI induce neuronal damage, reactive gliosis and proliferation of glia and neurons.

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388. A role of phosphorylation of CREB for neural progenitor cells in the SVZ and peri-infarct area

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Background and purpose: We noted that transcription factor phosphorylation of cAMP response element-binding protein (CREB) is associated with a proliferation of neural progenitor cells (NPC) in the subventricular zone (SVZ) after ischemia. We examined whether acute focal ischemia induces neurogenesis and the expression of phosphorylation of CREB in the ischemic area.
Methods: Middle cerebral artery occlusion (MCAO) was administered to mice by filament insertion for 45 mins and sacrificed at 24 h, 72 h and 7 day after ischemia. 5-bromo-2-deoxyuridine (BrdU) was used to label proliferating cells and immunohistochemistry was performed.
Results: The numbers of BrdU/doublecortin (DCX) in the SVZ and the peri-infarct area significantly increased than contralateral side at 72 h ($P<0.05$). Phospho-CREB/DCX double-labeled cells also increased in the SVZ at 72 h ($P<0.05$). However, a small numbers of phospho-CREB/DCX double-labeled cells were seen from 72 h to 7day in the peri-infarct area.
Conclusions: Our result showed that phosphorylation of CREB might play an important role in survival of newly generated NPCs in the SVZ and peri-ischemic area after MCAO.

399. RNAI-mediated downregulation of Beclin1 attenuates focal cerebral ischemic injury and enhances neurogenesis in rats

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Objectives: Because autophagy appears to be occurring after cerebral ischemia, we tested the roles of Beclin 1-dependent autophagic way in focal cerebral ischemia in the rat middle cerebral artery occlusion model (MCAO).

Methods: Lentiviral vectors-associated RNA interference (RNAi) system was stereotaxically injected into the ipsilateral lateral ventricle to reduce Beclin1 expression. We than evaluated the ipsilateral infarct volume, autophagosomes formation, neurogenesis and apoptosis which might be modulated by Beclin1 RNAi.
Results: Beclin1 RNAi not only inhibited the autophagosomes formation but also repaired the ischemic injury in the ipsilateral hemisphere. On the 14th day after MCAO, Beclin-1 downregulation by RNAi increased the number of neural progenitor cells, newborn immature and mature neurons and reduced the apoptosis of immature striatal neurons in the surrounding ischemic core of ipsilateral hemisphere.
Conclusions: RNAi-mediated downregulation of Beclin1 improves outcomes after transient MCAO. This neuroprotective effect is likely exerted by inhibiting autophagy and apoptosis and enhancing neurogenesis.

502. Modulation of fate determinants Olig2 and Pax6 in resident Glia evokes spiking neurons receiving synaptic input after mild brain ischemia

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Background: Although *in vitro* studies suggest that non-neurogenic regions of the adult central nervous system potentially contain multipotent parenchymal progenitors, neurons are clearly not replaced in most brain regions after injury. The transcription factors Olig2 and Pax6 play largely opposing roles in determining progenitor cell fate in neurosphere culture as well as in the adult mammalian central nervous system *in vivo*. We have previously demonstrated that after cerebral ischemia astrocytes expressing enhanced green fluorescent protein (eGFP) under control of the GFAP promoter predominantly adopt a complex electrophysiological phenotype. Importantly, the majority of GFAP−eGFP+ cells are also Olig2-immunoreactive. Therefore, we here explored Olig2 antagonism and Pax6 overexpression in a model of mild transient brain ischemia to re-direct endogenous resident progenitors proliferating *in situ* toward a neuronal fate.
Methods: 129/Sv wildtype mice were subjected to 30 mins of filamentous middle cerebral artery occlusion (MCAo) followed by reperfusion. 48 h later, retroviruses containing either only GFP, Olig2-VP16-IRES-GFP to interfere with Olig2 function, or Pax6-IRES-GFP were injected stereotaxically into the ischemic lesion core, i.e. the lateral striatum. Animals were killed at 10 and 17 days after MCAo for further immunohistological and electrophysiological analysis.
Results: Cells transduced with the control vector showed complex glial membrane properties and did not express Doublecortin (DCX). By contrast, DCX+ cells emerged reproducibly both after repression of Olig2 and after overexpression of Pax6. Similarly, after modulation with fate determinants cells expressed Na+ currents to a higher percentage at both time points after MCAo. After infection with the control virus cells did not elicit action potentials, but we were able to detect action potentials after repression of Olig2 and after overexpression of Pax6. Commonly, we observed a single action potential during the depolarizing pulse, but in some cases more than one action potential was observed. After Pax6 transduction we also observed spontaneous current events.
Conclusion: Therapeutic gene transfer via retroviral vectors into the ischemic lateral striatum resulted in a significant number of infected cells differentiating into immature DCX+ neurons with Na+ currents capable of generating single action potentials. Our study demonstrates that resident glia can be re-programmed toward functional neuronal differentiation following brain injury *in vivo*, including specifically synaptic integration. Our present data provide proof-of-principle evidence that resident cells proliferating in ischemic gray matter can be recruited into functional neuronal differentiation, a crucial first step toward neurorepair.

694. Baicalin can promote neuronal differentiation via modulating Jak/Stat3 and Mash1 pathways in rat neural progenitor cells

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Objectives: Baicalin, a flavonoid isolated from the root of Scutellaria baicalensis G, has been proved to protect neural cells from oxidative injury. Whether Baicalin could promote neurogenesis is unknown

yet. In this study, we hypothesized that Baicalin could stimulate neurogenesis by promoting the differentiation of neural progenitor cells. To verify the hypothesis, we investigated the effects of Baicalin on the proliferation and differentiation of neural progenitor cells.
Methods: Neural progenitor cells were prepared from cortex of embryonic E16 Sprague Dawley rats. The dissociated cells were seeded at a density of 1×10^5cells/ml in a DMEM/F12, replenished with 2% B_{27}, recombinant human basic fibroblast growth factor, and epidermal growth factor. The cells were then cultured with a differentiation medium contained DMEM/F12 (1:1) replenished 2% B_{27}, 5% fetal bovine serum for 14 days. Different concentrations of Baicalin (1, 2, 10, 20 mmol/L) were added into the cultured medium. The newly formed neurons were identified by co-staining with BrdU, anti-Tublin b-III (Tuj1), anti-microtubule associated protein-2 (MAP-2) and 4′,6′-diamidino-2-phenylindole (DAPI) whereas the formation of glial cells conformed by co-staining with BrdU and anti-glial fibrillary acidic protein (GFAP). Fluorescent imaging was visualized with a fluorescence microscope system (Leica DMIL). To explore the mechanisms of Baicalin promoting neuronal differentiation of neural progenitor cells, we investigated the expressions of p-Stat3 and Mash1 with western blot and Q-PCR analysis.
Results: Fluorescent studies showed that Baicalin treatment promoted the neural progenitor cells to differentiate into neurons but inhibited the formation of glial cells. Western blot analysis and Q-PCR study showed that Baicalin dose dependently down-regulated the expression of p-Stat3 but up-regulated Mash1.
Conclusion: Baicalin could promote neurogenesis instead of astrogliogenesis of neural progenitor cells, which is mediated by suppressing Jak/Stat3 pathway and activating Mash1 gene expression.
Acknowledgement: This study was support by a Seed Fund for Applied Research, University of Hong Kong and a Seed Fund for Basic Research, University of Hong Kong.

809. Dose dependence of isoflurane effects in the adult mouse brain

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Objective: The general anesthetic isoflurane has been shown to confer neuroprotection against ischemic insults by a number of mechanisms including enhancing hyperpolarization by GABA, increasing levels of anti-apoptotic proteins, and reducing glutamate release. On the other hand, isoflurane has been implicated in the generation and accumulation of Aß plaques found in Alzheimer's disease (AD) and apoptosis in the developing brain. To investigate mechanisms of isoflurane-induced neurological change, levels of doublecortin (DCX), a marker of neurogenesis, c-Fos, a marker of gene activation, HSP70, a marker of cellular stress, and Bcl-xL, an anti-apoptotic marker, were compared in adult mice exposed to various concentrations of isoflurane and recovered for different lengths of time after exposure.
Method: Adult, male CD-1 mice were exposed to 2 h of 0.6%, 1.3%, or 2% isoflurane anesthesia. After exposure, mice were given 2 h, 1, 6, or 14 days to recover. Immunohistochemistry involved staining for DCX, c-Fos, HSP70, and Bcl-xL. Blue fluorescence 4,6′-diamidino-2-phenylindole (DAPI) staining was used to identify cell nuclei. Doublecortin positive cells were assessed in the dentate gyrus. Cells positive for c-fos were counted in the cortex, amygdala, dentate gyrus, and CA1 region of the hippocampus.
Results: No cells positive for HSP70 or Bcl-xL were detected in any brain region. There were DCX positive cells found in the dentate gyrus in all animals. Whether DCX immunoactivity is significantly increased in the anesthetized animals has yet to be determined. Three-way ANOVA showed that levels of c-Fos were significantly different with respect to brain region ($P < 0.001$), the number of days after isoflurane exposure ($P < 0.001$), and doses of isoflurane ($P < 0.001$) and that there was a statistically significant interaction between region, day, and dose. The overall level of c-Fos expression in the dentate gyrus was lower than that observed in any of the other assessed brain regions. In the hippocampal CA1 region, dentate gyrus, and amygdala, c-fos expression was elevated at the day 0 time point. In the cortex, c-fos expression on day 0 was lower in the anesthetized animals than in the control animals.
Conclusion: Isoflurane anesthesia at sub-clinical and clinical concentrations caused neither cellular stress, as measured by HSP70, nor upregulation of anti-apoptotic genes, as measured by Bcl-xL, in the adult mouse brain. Isoflurane did affect the expression of c-Fos, an immediate early gene. The effects of isoflurane on c-Fos expression are dose dependent, time dependent, and region dependent. DCX positive cells found in the dentate gyrus after isoflurane exposure may indicate induction of neurogenesis and is worthy of further investigation.
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865. Canonical Wnt signaling promotes neurogenesis in the adult murine brain and reduces apoptotic cell death following focal cerebral ischemia

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Objectives: Canonical Wnt signaling is a conserved pathway during neural development.[1–3] It is essential for the expansion of the pool of neural progenitors[4] and in conferring neuronal identity.[5] Wnt signaling is also indispensable for hippocampal neurogenesis.[6] However, the role of Wnt signaling in promoting neurogenesis within the adult cortex has not been examined to date. With this work we aimed to determine the role of Canonical Wnt signaling in the context of:

(1) adult neurogenesis and
(2) neuroprotection following focal cerebral ischemia in mice.

Methods: 100 ng of purified Wnt-3a murine protein was injected intra-parenchymally into the cortex and striatum of 12-week old C57/Bl6 male mice. This was followed by daily administration of 5-bromo-2-deoxyuridine, BrdU, for one week followed by immunohistochemical analyses of the cell types present. Wnt-3a was injected in a similar manner 1 day after middle cerebral artery occlusion (MCAO), a model of focal cerebral ischemia, followed by BrdU administration for one week and immunohistochemical analyses, using activated Caspase-3 as a marker of apoptosis.
Results: When injected into the naïve adult cerebral cortex and striatum, Wnt-3a promoted *de novo* neurogenesis as indicated by the expression of GFAP, Nestin and Doublecortin. Cells positive for these markers were situated along the needle track and were also positive for the mature astroglial cell marker s-100β. In addition, there was a large pool of BrdU-positive IBA-1 (microglial) cells situated close to the neural progenitor cells.

Injecting Wnt-3a after MCAO resulted in a significant (6-fold, $P<0.0001$) reduction in apoptosis of the BrdU positive cells within the cortex and the striatum. This effect was also seen when Wnt-3a was injected prior to the insult, as well as up to 3 days after it and persisted at 6 months post-Wnt-3a and BrdU injection ($P<0.0001$).
Conclusions: Canonical Wnt signaling is able to induce *de novo* neurogenesis within the adult murine cortex and striatum, possibly by causing de-differentiation of mature astrocytes. The role of the pool of proliferating microglial cells is yet to be established. Both pre- and post-MCAO injection of Wnt-3a has a protective effect on the proliferating cells within the murine cortex and striatum.

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950. Regulation of post ischemic neurogenesis by IGFBP-3

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Objectives: Cerebral ischemia enchances the proliferation of neural progenitor cells in the neurogenic regions, while upregulating the expression of several growth factors including, IGF-1 and IGFBP-3 in the ischemic cortex. Although, IGF-1 increases the proliferation, differentiation and survival of neural progenitor cells, the role of Insulin like growth factor binding protein-3 (IGFBP-3) in the ischemic brain is not clear.

Methods: To understand the role of IGFBP-3 in the post ischemic brain, we analyzed the effect of IGFBP-3 on the IGF-1 mediated proliferation of neural progenitor cells (NPC) *in vitro*. We used cultured neural progenitor cells stimulated by the tested agents and measured cell proliferation, release of LDH, incorporation of BrdU and analysis of phospho-Akt and cyclin D1.

Results: The stimulation of neural progenitor cells with IGF-1 (50 ng/mL) and FGF2 (20 ng/mL) enhanced the proliferation of cells. Incubation of NPC's with IGFBP-3 (5, 50, 500 ng/mL) reduced the neurosphere formation only at 500 ng/mL. The decrease in the neurosphere formation was consistent with the decline in the metabolic activity of the cells, however IGFBP-3 did not cause cell death as measured by the release of LDH. Consistent with these observations, BrdU incorporation studies established a role for IGFBP-3 in the inhibition of cell proliferation. Furthermore, immunoblot analysis demonstrated a decrease in the content of phospho-Akt and cyclin D1 following incubation with IGFBP-3, suggesting an inhibitory role for IGFBP-3 in the IGF-1 mediated proliferation of cells.
Conclusions: These results together suggest that IGFBP-3 may play an important role in regulating post ischemic neurogenesis. These results may have wider implications for the modulation of brain repair processes after a variety of insults.

958. Role of IL10 in the neurogenesis of the postnatal SVZ of rodents

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Objectives: The subventricular zone (SVZ) is the main neurogenic area in adult brain and after experimental models of stroke. In normal conditions the neuroblasts generated in the SVZ migrate and differentiate into mature interneurons in the olfactory bulb. After ischemia these neuroblasts migrate to the lesion areas indicating that adult neurogenesis can be modulated by pathological situations.[1,2] The specific relevance of the inflammation associated to CNS pathologies in adult neurogenesis is unclear. The first studies showed that inflammation was detrimental for hippocampal neurogenesis but the most recent studies indicated that this context is much more complex.[3–5] The aim of this study is to explore the effects of IL10 in the differentiation of the neural stem cell (NSC) from the postnatal SVZ in normal and pathological situations.
Methods: Several primary cultures (dissociated, explants, neurospheres) from the postnatal SVZ were performed from postnatal rats. Cell viability, proliferation and differentiation were explored after interleukins treatment. In living cultures cell viability was measured by IdPr incorporation and cell proliferation was evaluated by a pulse of Brdu. Cellular markers (nestin, BIII tubulin, DCX, and others) were detected by immunofluorescence on fix cultures. By western blotting the intracellular pathways activated after interleukin stimulation were quantified. The studies were mainly focused in detect the intracellular activation of the Jak/Stat and MAPK pathways. By RT-PCR the levels of interleukins and its receptors were analyzed in cell culture and in *in vivo* samples. Histological analyses by immunofluorescence and western blotting of different cellular markers were evaluated in neurogenic and non neurogenic areas of the adult mice.
Results: The postnatal SVZ express high levels of IL10 receptor. On primary cultures of the SVZ the exposure to IL10 stimulates the phosphorylation of STAT 3 as well as ERK1/2 p42-p44 (pErk). The inhibition of the MAPK pathway abolished the phosphorylation of Stat3. This observation indicates that pERK is upstream of STAT3 activation after IL10 stimulation. Double immunofluorescences techniques show that pERK is specifically activated on NSC (Nestin +). The presence of IL10 on primary culture of SVZ for several days induces cell death but have not effects on cell proliferation. In these cultures Il10 induces changes in the number of NSC. Preliminary *in vivo* studies in IL10 KO mice indicate that the neurogenesis in the SVZ is altered.
Conclusions: The present results show that NSC respond to IL10 by activation of ERK 1/2 that induces the phosphorylation of STAT3, and suggest that this anti-inflammatory cytokine plays a role in the NSC differentiation.
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1023. Agmatine enhances neurogenesis of transplanted striatum-derived neural stem cells in rat brain after experimental stroke

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Objectives: Transient ischemic attack (TIA) has long been identified as a risk factor for stroke. Ischemic injury can cause massive damage from extensive tissue loss, following the neuronal cells and the connections that reside there. Agmatine has been shown to have neuroprotective effect and ability to modulate differentiation of neural stem cells in various neuronal disease models.[1,2] To better repair following stroke injury, stroke damaged animal models were transplanted with striatum-derived neural progenitor cells and treated agmatine in this study.

Methods: Using a well established models of focal cerebral ischemia, male Sprague-Dawley rats (300 ± 30 g) were subjected to 60 mins middle cerebral artery occlusion (MCAO).[2] Before transplantation, striatum-derived neural progenitor stem cells isolated from E14 fetal ICR mice brain were formed undifferentiated neurospheres *in vitro* stained by BrdU. Animals were transplanted with striatum-derived neural stem cells at 1 week after MACO injury and agmatine was co-treated for 7 days to 21 days at the end of occlusion (100 mg/kg, I.P.). Animals were pretreated by agmatine after the injury was also transplanted with the neural stem cells at 1 week. To analyze behavior performances, rotarod test, limb placing test were executed after MCAO. To investigate whether agmatine involved the differentiation and proliferation of striatum-derived neural progenitor cells, double immunohistochemical staining was performed with anti-BrdU antibody for donor-derived cells, neuronal marker NeuN and MAP2, migrating neuroblast marker DCX, and astrogial marker GFAP antibodies.

Results: Pretreatment of agmatine helped the neural progenitor cells differentiate to become mature neural cells at 7 days to 14 days after transplantation. Histological analysis supported that implantation of the striatum-derived neural progenitor cells with agmatine promoted functional recovery, and decreased infarction cavity and scar tissue formation compared to lesion-control group. The striatum-derived neuronal progenitor cells transplanted groups with agmatine regained approximately normal reflex in response to the stimuli within 3 weeks. In contrast, lesion-control group regained only about 50% of reflex response for 4 weeks.

Conclusions: Here, we show that agmatine which has been known as a neuromodulator in the brain enhances neurogenesis of striatum-derived neural stem cells. These results displayed that cell derived from neural progenitor cells exhibited the differentiation of functional neurons and synapse formation by agmatine treatment.