Poster Session 2: Functional recovery

P I - 2-1

The Amyloid Precursor Protein family - distinct modulators of neuronal injury

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Question: The Amyloid Precursor Protein (APP) is intensely studied due to its intimate connection to Alzheimer"s disease. APP is part of a gene family with Amyloid Precursor Like Protein 1 (APLP1) and Amyloid Precursor Like Protein 2 (APLP2). The APP-family are associated with a range of neuronal activities, including neuroprotection, neuritogenesis and cell development. This study tested the role of APP and APLP2 in modulating neuronal injury following different clinically relevant insults, and if its actions are sex-dependent.

Methods: Cuprizone demyelination/remyelination model: APP and APLP2 knockout mice (APPko, APLP2ko), both sexes, were treated with the demyelinating agent cuprizone in the diet for 2 weeks (demyelination phase), and then normal chow for 2 weeks (remyelination phase). Myelination was measured by transmission electron microscopy and immunohistochemically.

Motor neurone disease (MND) model: The G37R:SOD transgenic mouse model (encoding G37R mutants superoxide dismutase) was crossed with either APPko or APLP2ko mice. The APPko:G37RSOD, APLP2ko:G37RSOD and G37RSOD mice (both sexes) were evaluated for motor function by rotarod and DigiGait. Immunohistochemistry was performed on brain, spinal cord and muscle tissue to assess pathological changes.

Traumatic brain injury model: Rats (Sprague-Dawley, male), or APPko and APLP2ko mice (both sexes) were injured by either the weight drop method or the closed cortical impact model. Motor function was assessed by either rotarod or DigiGait. Immunohistochemistry was performed on brain tissue to assess pathological changes.

Statistical analysis. Statistical analysis of differences between animal groups was performed using one-way or two-way analysis of variance with the Tukey post hoc-test.

Results: We found that APP and APLP2 expression significantly modulated different aspects of neuronal injury in rodent models of traumatic brain injury, demyelination and motor neurone disease. Some of these effects were found to be sex and/or age dependent. In the cuprizone demyelination/ remyelination model, the myelinated callosal axons in APPko mice were less susceptible to cuprizone-induced demyelination and showed a failure in remyelination after cuprizone withdrawal when compared to wildtype and APLP2ko mice. In the motor neurone disease model the female APLP2ko:G37RSOD mice had an increased life span compared to the male APLP2ko:G37RSOD. We also observed sex dependent significant differences in survival for the APLP2 heterozygous knockout mice indicating gene dosage effects. In traumatic brain injury we observed divergent effects, with APPko performing worse and APLP2ko performing better on motor function tests. An effect that was sex-dependent.

Conclusions: These studies demonstrate the APP-family members APP and APLP2 affect functional and pathological outcomes in distinct, clinically relevant, neuronal injury models in a sex and gene-dosage dependent manner.

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Rapamycin enhances mitophagy and attenuates apoptosis and oxidative stress after spinal ischemia-reperfusion injury

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Question: The spinal cord is extremely vulnerable to ischemia-reperfusion (I/R) injury, and the mitochondrion is the most crucial interventional target. Rapamycin can promote autophagy and exert neuroprotective effects in several diseases of the central nervous system. Therefore, we hypothesize that rapamycin may work though mitophagy stimulation to protect mice with spinal cord ischemia-reperfusion (SCIR) .

Methods: Male mice underwent aortic arch distal occlusion 10 minutes to produce SCIR injury. We examine whether rapamycin treatment promotes mitophagy and reduces neurons apoptosis, oxidative stress and locomotor impairment after spinal cord ischemia-reperfusion injury (SCIR) in mice.

Results: We found that rapamycin significantly improved locomotor function in SCIR mice between 24 hours and 7 days of recovery. At 24 hours following SCIR, rapamycin markedly enhanced mitophagy via increasing the translocation of p62 and Parkin to the damaged mitochondria. Strikingly, it reduced neuronal loss and apoptosis in the injured spinal cord. Rapamyicn depressed apoptosis by inhibiting Bax translocation to mitochondria and cytochrome c release from mitochondria. Moreover, rapamycin significantly decreased mitochondrial oxidative stress.

Conclusions: Our results demonstrate that rapamycin promotes mitophagy and reduces SCIR-induced neuronal apoptosis, indicating its potentially therapeutic application against SCIR.

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Identification of early electrophysiological markers to predict spontaneous post stroke recovery in mice

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Objectives: Clinical and preclinical studies in the field of stroke recovery are limited by the large variability of the acute motor deficit and subsequent spontaneous recovery. In particular, proportional recovery has been observed in many clinical studies but in a subset of patients, recovery is not proportional to initial impairment and is often poor. Identification of these outliers is crucial in the design and interpretation of stroke rehabilitation trials. In this study we exploit a mouse model of stroke to identify early electrophysiological markers/changes that are related with spontaneous recovery, thus providing a reliable base to predict immediately after stroke the extent of potential motor improvement that each subject will show over time.

Materials and methods: Permanent Middle Cerebral Artery Occlusion (MCAO) was used to induce an ischemic insult in the right hemisphere in mice. Chronic bipolar Local Field Potentials (LFPs) were recorded from the perilesional and contralesional Caudal Forelimb Area (CFA) before stroke and 2, 16 and 30 days post-stroke. LFPs were recorded both in freely moving condition and during a goal-directed retraction task, performed on a robotic

platform able to record kinetic and kinematic features of the retraction movement (e.g. force exerted, time to reach the goal, positions, number of force peaks, etc.). Gridwalk and Skilled Reaching test were used to evaluate the motor performance at 2, 9, 16, 23, and 30 days post lesion. To determine the relation between electrophysiological biomarkers and spontaneous motor recovery we performed a linear correlation analysis.

Results: In naïve animals the Power Spectral Density (PSD) analysis during the retraction task, revealed a consistent increase of Theta band, together with a reduction of Beta and Gamma bands during active phase relative to the resting state. Interestingly, stroke reduces these differences between active and resting state in the perilesional area but not in the contralateral hemisphere. Moreover the peak-to-peak amplitude of the Event Related Potential (ERP) aligned on the start of the retraction movement shows a decrease in the acute post-stroke phase in the ipsilesional hemisphere but not in the contralesional side. This impairment in ERP amplitude partially recovers 1 month later. We next tested whether the acute neurophysiological changes in terms of PSD and ERPs amplitude during retraction task and PSD during freely moving recordings, are good predictors of spontaneous recovery. Interestingly, acute changes in specific spectral bands correlate with the post stroke deficit but also with the spontaneous recovery observed at 1 month both in general motor tests (Gridwalk and Skilled Reaching tests) and on the robotic platform.

Conclusions: Analysis of LFP spectral bands and movement-related ERPs in the acute post-stroke phase hold promise as relevant biomarkers to predict the extent of recovery from upper-limb impairment.

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Adaptation of tape removal test for sensation measurement in perineal area of rat

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Regeneration after spinal cord injury is a goal of many studies. Though the most obvious target is to recover motor function, sensory regeneration can improve quality life in paraplegic patients. According to a patient's survey, recovery of sensation in the perineal and genital area is one of the highest priority. Although there are behavioural tests to measure sensitivity, there is no test for measuring sensation in perineal and genital area.

The aim of our study was to develop the behavioural test for measuring the sensitivity of perineal and genital area in rats. We have modified the tape removal test used routinely to test sensorimotor deficits after stroke for the study. We adapted the test to perineal area and tested several settings. A small piece of tape (approximately 1 cm2) was attached to perineal area on the left side. Time to first touch and to remove the tape was measured. If the rat didn't remove the tape until 5 minutes the tape was removed and the time of 5 min was recorded. This was repeated 4 times for each animal with 3 min pause between each trial. Each rat was trained in 5 consecutive days and then tested weekly. We tested different rat strains (Wistar, Sprague-Dawley, Long-Evans and Lewis), shaving and non-shaving and different types of tapes. All tests were performed on healthy animals and animals with T10 dorsal hemisection lesion. After the lesion animals were tested at day 3, 8, 14 and 21.

We found that the test was suitable for all tested strains, however, the Lewis rats achieved the lowest contact times. But this difference was significant only in first few days of learning the task. There were no significant differences between gender and different types of tape or shaving. After the animals underwent dorsal hemisection spinal cord injury the test could detect sensory deficit, the time to sense the stimulus increased from 1"32 up to 3"20 in average. This was compared with other behavioural tests (BBB, von Frey, ladder and Plantar test).

We conclude, tape removal test is suitable for testing perineal and genital sensation in rats and can be used in different strains.

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Triiodothyronine modulates mechanisms of brain plasticity after experimental stroke

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Aims: Thyroid hormone triiodothyronine (T_3) is the active form in the adult brain and may regulate several mechanisms of neurorepair. The aim of this study is to investigate long-term effects of T_3 on mechanisms that may stimulate synaptic plasticity and promote functional recovery after experimental stroke.

Methods: Forty-four male B6/C57 mice were randomly distributed into surgery groups and subjected to Photothrombosis (PT) for 20 minutes or Sham operation. Baseline sensorimotor function was assessed by the rotating pole test (rpt) before surgeries. For dendritic spine analysis, Thy1-YFP transgenic mice were subjected to PT. On day 2 after PT, animals were randomized into treatment groups with T_3 (i.p. 50 µg/kg) or vehicle (saline; i.p.) and treated every second day for 12 days. Fourteen days after PT, neurological deficits were assessed by rpt,

brains were collected for infarct volume, immunoblotting, immunohistochemistry and dendritic spine analysis. Spines were automatically detected using NeuronStudio Software and classified according head to neck ratio and head diameter as stubby, mushroom or thin.

Results: Treatment with T_3 significantly enhanced functional recovery of lost neurological functions, without differences in the infarct volume between groups. Importantly, T_3 administration did not affect physiological parameters including body weight or temperature. Interestingly, treatment with T_3 reduced the number of parvalbumin$^+$ /c-fos$^+$ neurons, decreased glutamic acid decarboxylase 65/67 levels and increased dendritic spine density in the peri-infarct motor cortex.

Conclusions: T_3 modulates mechanisms that promote structural plasticity after experimental stroke and our results might be exploited in future clinical stroke trials.

P II - 2-6

Significant neuroprotective activity of the small molecule #117 in a mouse model of photothrombotic focal ischemia when given up to 12 hours post-injury

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Introduction: GHB (γ-hydroxybutyric acid) is an endogenous compound found in the mammalian brain, which is structurally related to GABA. GHB possesses unique high-affinity binding sites in the postnatal forebrain with undisclosed functional significance. Pre-clinical studies have shown promising effects of GHB as a neuroprotective agent following ischemia. In this study, we investigated the neuroprotective potential of an in-house developed GHB analogue named #117, which is brain-permeable after systemic administration (B/P 0.37), and superior to GHB in terms of affinity (50 times higher than GHB for the specific binding site), and selectivity (i.e. no affinity for $GABA_B$ receptors or 45 other neurotargets).

Methods: For induction of a focal ischemic damage to the left motor cortex we used the photothrombotic model of stroke in male C57BL/6 mice. Infarct volumes were quantified three days post-injury using cresyl violet staining and image processing. Motor performance was evaluated three days post-injury using the grid-walk and cylinder asymmetry tests. Mice were grouped 5-7 per condition and all observers were blinded to the treatment groups.

Results: When compound #117 (175 mg/g i.p.) was administered 30 min, 3 hrs or 12 hrs after the injury and compared to saline-treated animals, we observed significant effects on both infarct size and motor performance. Quantification of infarct volumes revealed significant reductions of 30% at 30 min (***P<0.0005), 71% at 3 hrs (**P<0.01), and 46% at 12 hrs (**P<0.01). These effects were accompanied by significant improvement of motor function and a decrease in the expression of selected inflammatory markers (CD14 and MMP9).

To begin to address the mechanism-of-action of compound #117 under ischemia *in vitro*, we have investigated the effect of compound #117 in cultured hippocampal neurons exposed to L-Glu-induced excitotoxicity. We have found that 1-5 mM of the compound is able to promote cell survival after excitotoxicity with 138% (*P<0.05).

Conclusions: The small molecule #117 demonstrates significant neuroprotective properties in a pre-clinical model of stroke. This underscores a potential of the compound for the treatment of acute brain injury in general. The favourable physico-chemical properties, brain penetration and tolerability (no apparent toxic or sedative effects) of the compound furthermore underscores its clinical potential. The intriguing effects observed after 3

or 12 hours of compound administration suggests a mechanism-of-action related to the sustained ischemic phase after the injury, possibly reducing the amount of cell death and inflammation. Further molecular and cellular studies are currently ongoing in our labs to link the neuroprotective activity of #117 to its molecular target.