**Institute of Experimental Medicine AS CR, Prague**
**Centre for Reconstructive Neuroscience (NeuroRecon)**
**Projects and People: March 2017**

## Project 1. Are perineuronal nets the long-term scaffold for memory?

**Jessica Kwok, James Fawcett, Vincenzo de Paola (Imperial), Giovanna Mallucci (Cambridge), Pavla Jendelova, Jiri Ruzicka**

The long-term coding of memory is not understood. Intriguingly, memories are retained after general anaesthetic, hibernation and ischaemia, all of which lead to withdrawal of the presynaptic terminals. Is there a structure which guides these synapses to re-form in the correct places? A few years ago Roger Tsien published a speculative article in which he hypothesized that the extracellular matrix, and particularly perineuronal nets, are the encoders of memory. He started to investigate this idea before he died, investigating the idea that PNNs might be modifiable during memory events by proteolysis. PNN digestion does not erase memories, but it is possible that PNNs might guide synapses back to the right place after withdrawal due to ischaemia or hibernation.

The concept for the project is to test the hypothesis that PNNs might be a scaffold for restoring memory after ischaemia and/or hibernation Two types of experiment are planned.

1) Testing memory: Animals will learn a long-lasting memory. Possible tasks are fear conditioning memory and memory of the position of a refuge platform in the water maze. They will then receive an injection of chondroitinase or hyaluronidase into the relevant brain region. Some animals will then have synapse withdrawal induced by anoxia or hibernation. Finally, memory will be tested again to see if the PNNs were necessary for memory restoration.

2) Imaging of synapse re-formation: The Mallucci lab has shown that synapses withdraw with hibernation and remake their connections when the brain temperature is restored. The question is whether PNNs guide these synapses back to the same place. Synapses and PNNs will be imaged in the de Paola lab. The first experiments will be in brain slices, with whole animals later.

## Project 2. Integrins and biomaterials in the spinal cord
## Sarka Kubinova, James Fawcett, Jessica Kwok, Kristyna Zaviskova;

Biomaterials have great potential for filling and bridging spinal cord injuries, but to date the amount of axon regeneration into a range of materials has been disappointing. This project will examine two possible reasons for this:
1. There is a mismatch between the receptors on axons and the ligands in grafts.
2. Integrins in axons are inactivated by inhibitory molecules in the CNS environment.

The project brings together expertise in Prague on the design of biomaterials which development of integrin-mediated sensory regeneration in Cambridge and analysis of ECM changes after injury in Leeds.

The overall concept is that it will be possible to obtain profuse growth of sensory axons into biomaterials grafts in the spinal cord by ensuring that the grafts contain an integrin ligand, by ensuring that the sensory axons express the relevant integrin, and by preventing integrin inactivation by expression of the activator kindlin-1.

The first step is *in vitro* testing of growth from adult sensory ganglia onto a range of biomaterials, to which integrin ligands have been incorporated.

The outcome of the experiments will be an analysis of the regeneration effects of matching integrins, integrin activation and biomaterials properties. Hopefully profuse sensory regeneration into and through the grafts will be seen.

## Project 3. The effect of 4MU on memory
## Pavla Jendelova, Jessica Kwok, Sujeong Yang, James Fawcett, Jana Dubišová

The project aim is to test a potential pharmaceutical that acts on perineuronal nets as a treatment for memory loss.
Perineuronal nets (PNNs) are important for the control of neuronal plasticity and memory in the adult brain. Removal of PNNs prolongs memory retention in mice, and corrects memory deficits resulting from Alzheimer's models and ageing.
We need a pharmaceutical that can be given to patients with memory deficits. We have recently shown that oral administration of 4MU (an inhibitor of hyaluronan synthase) is effective in the downregulation of PNNs in the spinal cord, and 4MU is a registered drug for human use. The question we aim to answer is – can we prolong and restore memory in rodents using 4MU?

We shall first determine the optimal dosage and side effect of 4MU in rodents, followed by the effect of 4MU in memory retention.

Are there any side effects?*:* The main target of 4MU is hyaluronan (HA), which is also present in other tissues including cartilage, kidney, glycocalyx in blood vessels and during inflammation. The compound is licensed at a lower dose for shorter treatments, so a long-term effects study is needed.

Effects on Memory retention: Animals will be treated with 4MU at an optimal dosage which was determined from the first objective. At a time point when PNNs are down-regulated, the animals will be subject to different memory tests, including novel object recognition, Morris water maze, object placement recognition tests. If this is successful, the ability of 4MU to restore memory in Alzheimer's and ageing will be tested.

## Project 4. Sensory reconstruction with recovery of perineal and genital sensation

**Pavla Jendelova, Sarka Kubinova, James Fawcett, Katerina Neumannova, Lucia Machova-Urdzikova**


The project is a follow- on from the demonstration that the sensory pathway in the spinal cord can be reconstructed after dorsal root crush by expression of alpha9 integrin (tenascin-binding) and the integrin activator kindlin-1 in sensory ganglia.

The aim is to restore sensation after spinal cord injury. The focus will be on lumbar DRGs, with the aim of restoring perineal and genital sensation, which is the highest priority for spinal injury patients.

The specific aims will be:

1. Develop sensory tests for the rat perineum and male genitalia
2. Enable re-innervation of the cuneate and gracile nuclei by regenerating sensory axons by removing perineuronal nets and possibly expressing NT-3
3. Test for sensory axon regeneration after spinal injuries made close to and far from alpha9/kindlin-transduced sensory neurons.
4. Changes in DRG gene expression after central axotomy with and without alpha9/kindlin.
5. Full experiment with behavior of optimized sensory regeneration protocol.

## Project 5. Neuroprotection in ALS

### Serhiy Forostyak, Slaven Erceg, Miroslava Anderova, James Fawcett, Jessica Kwok Dayanithi Govindan, Andras Lakatos (Cambridge), Monika Seneklova

Motoneuron death in ALS involves glia as well as the neurons. Damaged neurons secrete an unidentified signal that activates microglia, and these cells then activate astrocytes in the neurotoxic A2 type. The astrocytes then secrete an unidentified toxin to kill the neurons. The project will take microglial and astrocyte approaches to protection of motoneurons.

Astrocytes: Conversion of astrocytes to the A2 phenotype is clearly toxic. However some astrocyte types, particularly astrocytes derived from embryos, are very protective. The project will assay a variety of astrocyte types, using support of motoneurons in vitro as an assay. The aim is to find protective astrocyte types, characterize them, and work out how to deploy these cells in ALS models.

Microglia: Microglia are very migratory and interact with the extracellular matrix. A recent finding is that they produce large amounts of the matrix molecule ostoepontin and express integrins that allow them to interact with this and another injury molecule tenascin. They also produce a very active signaling PI3kinase. The project will look at the effects of these integrin and signaling effects and find out whether blocking the pathways will reduce the tendency of microglia to initiate neuronal killing.

## Project 6. Acoustic plasticity

### Josef Syka, Jessica Kwok, James Fawcett, Rosta Tureček, Jana Burianova

The Syka laboratory has developed a model of abnormal auditory development and plasticity after an early acoustic trauma event. They have also identified fast and slow ageing mouse strains. The aim will be to examine the role of perineuronal nets and associated molecules in these events.

## Project 7. Enhancing the migration and integration of stem cells for protection of motoneurons in ALS

### Serhiy Forostyak, Jessica Kwok, James Fawcett, Monika Seneklova

Stem cells and progenitors transplanted into the spinal cord can protect motoneurons in ALS models. However, these cells may not migrate far from their site of injection and may not make intimate contact with motoneurons. The aim is to enhance the migration ability of one of these progenitor cell types by transfecting with the tenascin-binding integrin alpha9 and with the integrin activator kindlin-1. This combination has enabled sensory axons to grow for

long distances in the cord, so it should be effective in promoting migration and integration of transplanted cells.

## Project 8. Proteoglycans in the CSF as markers of ALS progression
## Serhiy Forostyak, Jessica Kwok, Andras Lakatos, Axel Sandvig (Trondheim)

Perineuronal nets are destroyed in the rat SOD1 ALS model, and the proteoglycans from them are then found in the cerebrospinal fluid. Preliminary results suggest that the same may be true in human ALS patients. The project will examine CSF samples from human ALS patients, to determine whether proteoglycan levels could be a marker for disease progression and activity.