A CELL BIOLOGY APPROACH TO NETWORK DAMAGE AND RECOVERY IN BRAIN LESIONS

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Lesions to the central nervous system are a network problem: they not only affect the brain, but also spread to the wider system, to remote areas that the primary lesion site is functionally connected to. Indeed, a lesion triggers a number of degenerative phenomena that affect normal apoptosis and which are mostly associated to cell reactions to axonal loss and input deprivation, which finally result in remote cell death or degeneration. Most of the recovery mechanisms after the lesion (e.g., a stroke) occur on such remote areas, while brain tissue cannot be easily regenerated, so studies in this area are important for understanding the treatments that may be most effective for such recovery.

The aforementioned degenerative phenomena do not occur immediately after the lesion. On the contrary, they develop over time and have different timings, in terms of onset times, duration and timing of peak impact (i.e., the time where they are the most active), which can range from hours to months. Hence, remote sites that were originally not (severely) affected by the lesion may suffer from these effects over time.

Fortunately, these different timings may offer a time window for therapeutic interventions that, in turn, might be able to stop or reverse them, possibly markedly improving recovery times and decreasing the impact of the lesion to the whole nervous system. For this, it is important to study and understand the mechanisms that mediate remote cell death before we can stop or reverse its effects.

In order to get information about functional connectivity, we can look at images of the brain of the patient and compare it to control subjects, as well as to the contralateral side to where the lesion occurred, e.g., through fMRI. For example, this allowed to establish a model to study effects of a lesion far from the primary site of the insult on the cerebro-cerebellar circuit (i.e., the circuit that connects the motor cortex to the cerebellum) on humans. However, in order to study remote cell death mechanisms at the cellular level, we need to look into animal models, such as rodents. Then, we can use different markers, such as nitrergic signalling or cannabinoids receptors (e.g., CB2R), to assess the changes that are produced as a result of the lesion.

One of the mechanisms through which degeneration of remote areas occurs is autophagy, the system within the cells that tries to keep them clean (mainly through the intervention of the mitochondria). Under normal circumstances, autophagy markers keep the brain cells clean. However, they can also destroy them if the mechanism is disrupted. Autophagy is vital to the survival of the brain. Experiments with mice have shown that autophagy is disrupted in axotomised pre-cerebellar neurons due to mitochondrial dysfunction after a cerebellar lesion. Rapamycin can protect from damage by stimulating autophagy, thus promoting functional recovery. This discovery has therapeutic significance, and shows the potential of pro-autophagy treatments for acute brain pathologies, e.g., stroke and brain trauma.

Another mechanism associated with a better survival of neurons in remote areas is the activation of the nNOS enzyme after the lesion. Studies by Dr Molinari's group have shown the interactions between the cannabinoid signalling paths and nitrergic systems on rodents, and demonstrated that the increase of nNOS in neurons and decrease of iNOS in astrocytes improves molecular and, thus, neurological outcomes after cerebellar ablation. In contrast, the inhibition of nNOS activity affects in

a negative manner the neuronal survival, NO stress and neurological improvement, which is consistent with the known neuroprotective function of nNOS-derived NO signalling in axotomised neurons. Pharmacological blocking of iNOS in astrocytes has beneficial effects on neuronal survival, which implies that iNOS-derived NO from these cells is cytotoxic to injured neurons.

Neuroinflammation also occurs after the insult, resulting cell death at remote sites. This is associated with glial activation. Even though treatments for inflammation (e.g., treatment with methylprednisolone sodium succinate, or MPSS, which inhibits the immune response and blocks glial activation) exist, they do not fix the ultimate problem: once the treatment is discontinued, glial activation, and thus, cell death, is resumed.

So far, most of the research in this area has been done on rodents, e.g., mice. For example, the use of rapamycin on injured mice has demonstrated the potential of pro-autophagy treatments for acute brain pathologies, such as stroke and brain trauma. Even though it is expected that some of the results with drugs will be translated to humans, further research is needed in this area. Steps towards this path should be encouraged, especially regarding interactions between different drugs.

Regarding stimulation, Transcranial Magnetic Stimulation (TMS) has been used as a non-invasive tool to investigate the brain plasticity changes resulting from stroke and as a therapeutic modality to safely improve motor function. Remote cell death induced in rodents through the administration of HCb in the primary lesion site has been reduced by means of TMS. Still, there are many factors which remain unknown. For example, the large size of the coil with respect to that of the mouse implies that it is in practice very hard to define what part of the animal was being stimulated, and where is such stimulation most effective. For this, extensive research should be conducted to optimize the way repetitive TMS is utilized to reduce remote such cell death. Once these issues have been thoroughly studied, another path for possible future work might consist of acting on the cellular mechanisms not only through drugs, but also through neuromodulation.

Last, but not least, what we find in animal experiments is not directly implantable in clinics even after proper research on humans has been conducted. Thus, an interdisciplinary team is needed to feed back the research into clinical practice.