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Strokes and traumatic spinal cord and brain injury

MODULATION OF THE PERINEURAL NETWORKS USING PHYSICAL EXERCISE AFTER SPINAL CORD INJURY IN ANIMAL MODELS: PLASTICITY VERSUS STABILITY OF THE NEURAL CIRCUITS

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1. Summary

Spinal cord injury (MS) leads to significant deficits below the injury, which are often very disabling for affected patients; unfortunately, there are no treatments to reverse them. Physical rehabilitation is a promising strategy to modulate plasticity and promote functional recovery in these patients. Knowing their mechanisms of action and limitations would make it possible to optimize clinical protocols and design combination therapies that would improve the quality of life and recovery of these patients. Since perineural networks (PNNs) regulate neural plasticity, favoring synaptic stabilization versus plasticity, they are likely to be key in the plastic changes observed after an SCI. It is interesting to note that activity reduces brain PNN, favoring plasticity, but increases spinal PNN, enhancing the stability of spinal circuits. However, the role of PNNs after spinal cord injury and how rehabilitation could modulate them has been poorly studied. The present project is based on the hypothesis that, after SCI, the lower activity below the lesion would lead to a reduction in PNNs around the spinal motor neurons, which have lost innervation of the superior motor centers. This reduction facilitates disorganization of the spinal circuits, contributing to spasticity and neuropathic pain, two phenomena that occur after spinal cord injuries in the context of poorly adaptive plasticity. While physical rehabilitation increases the activity of motor neurons, consequently attenuating the loss of PNNs, it would limit the disorganization of the spinal circuits and, therefore, maladaptive plasticity. In contrast, an increase in PNNs could hinder the formation of new neural connections.

To evaluate the role of PNNs and activity in modulating plasticity, we used an experimental model of SCI in both wild-type and transgenic mice with vestigial PNNs (due to lack of Link1 protein, an important component of these networks), and we assessed how exercise modulates these PNNs after SCI. We also assessed whether these PNNs limit the formation of new connections between denervated motor neurons and the injured corticospinal tract. A better knowledge of the effects of physical activity on PNNs after an SCI would make it possible to design strategies that strike a balance between enhancing stabilization of the spinal circuits to prevent poorly adaptive plasticity but at the same time promoting the regeneration of injured or spare axons to improve functional recovery.

The objectives addressed in the project were:

1- To evaluate how physical activity modulates the plastic changes induced by SCI, focusing on the role of the PNNs of the spinal motor neurons below the injury, relating these changes to poorly adaptive plasticity and functional recovery.
2- To evaluate the role of spinal PNNs in motor function, using transgenic mice that do not have vestigial PNNs
3- To evaluate the inhibitory role of PNNs in the growth of collaterals from the descending corticospinal tract to spinal motor neurons after SCI, focusing on the role of Sema 3A in this inhibition.
4- Evaluate the role of PNNs in the cervical spinal cord, focusing on the respiratory circuits and how the injury can alter these PNNs and modulate their plasticity.

2. Results

Role of PNNs and physical activity in modulating SCI-induced maladaptive plastic changes

In an animal model, we assessed the impact of a thoracic SCI on the lumbar spinal motoneurons (below the lesion) and observed that there was a reduction in the PNNs surrounding them. In addition, by electrophysiological techniques, we observed an increase in spinal reflexes (hyperreflexia) and to thermal pain (thermal hyperalgesia). If animals with SCI were subjected to different types of physical activity (forced to run an hour on a treadmill, living in an enriched environment, or having 24-hour access to an exercise wheel), the reduction of PNN induced by the SCI was attenuated or even reversed. Moreover, 24-hour wheel activity was much more effective than enrichment or 1h/day treadmill running in order to preserve PNN and attenuate hyperreflexia, and a correlation was observed between the degree of physical activity performed in the wheel and the degree of preservation of PNN. In contrast, both types of exercise (wheel and treadmill) reduced the thermal hyperalgesia that was developed after the injury. The results confirm that physical activity increases spinal PNNs and thus promotes the stabilization of circuits and limits the poorly adaptive plasticity observed after SCI.

Characterization of a transgenic mouse with vestigial PNNs to assess the role of spinal PNNs in motor function

We used a transgenic mouse with vestigial PNNs due to the lack of one of its components, the Link1 protein or Crtl1 (Crtl1 KO). The initial aim of the study was to compare the effects of SCI and rehabilitation in these transgenic mice, in order to investigate the role of spinal PNNs after SCI. First, however, we wanted to characterize the model at baseline. To our surprise, we observed that these mice exhibited subtle but very revealing motor alterations. Crtl1 KO mice had PNNs with alterations in their components in both the motor cortex and the spinal cord. Behavioral and electrophysiological tests revealed motor deficiencies, such as hypoactivity, inability to run on the treadmill at the same maximum speed as wild type mice, and alterations in motor coordination. In addition, they showed hyperexcitability of spinal reflexes and impaired motor unit recruitment. These functional outcomes were accompanied by an increase in excitatory synapses around spinal motoneurons. There was also a reduction in the number of larger motoneurons, probably because the higher excitatory synaptic input favors the development of a profile of smaller, more excitable motoneurons. The results denote the importance of ensuring stable spinal circuits, at maturity, for proper motor function. Alteration of spinal PNNs promotes an excess of excitatory synapses and induces spinal hyperreflexia that is similar to that seen after spinal cord injury. In fact, transgenic mice at the basal level have features reminiscent of SCI mice.

Since we already showed in the first objective that there is a disorganization of the PNNs below the injury after an SCI, and that their preservation through physical activity helps to reduce hyperexcitability, this study confirms the importance of preserving PNNs to ensure a proper motor function, as well as the relationship between PNNs, the stability of spinal circuits, and maladaptive plasticity. Unfortunately, the basal alterations of these mice at the motor level are also a handicap to study the impact of post-SCI rehabilitation on this model. Since they are hypoactive and fail to run on a treadmill at the maximum speeds reached by wild type mice, the intensity of physical activity that they can achieve will always be lower than for wild type animals, making it difficult to compare the effects of rehabilitation on these mice.

Inhibitory role of PNN in the growth of collaterals from the descending corticospinal tract to spinal motoneurons after SCI

The transgenic mice with vestigial PNN also have lower expression of Sema3A in these nets, Sema3A being an important inhibitory component. Therefore, we explored the role of these networks in limiting the plasticity of the corticospinal tract after injury. After injuring the right corticospinal tract we explored the ability of the contralateral (intact) tract to make collateral innervation, a phenomenon that occurs spontaneously but to a very limited extent after partial injuries, in an attempt to compensate for the loss of the function of the other tract. As expected, the fact that the spinal motor neurons have vestigial PNNs made the spinal cord more permissive for the axons of the corticospinal tract to collaterally innervate the denervated area. Therefore, we can conclude that the lack of Crtl1 generates aberrant PNNs that lead to altered PNNs, which cannot guarantee the optimal stability of the spinal circuits, but this disorganization generates a permissive scenario for the growth of contralateral axons after injury.

Impact of spinal cord injury on cervical PNNs involved in respiratory tract

Because PNNs have differential modulation depending on the anatomical region where they are located, we wanted to explore the behaviour of PNNs that surround the neurons from the respiratory network, extremely plastic and crucial for a vital function such as breathing. Although these circuits are located in the spinal cord as the circuits that control locomotion, they are where the internal and highly reflective regulation component predominates, and could have a different regulation in terms of stability and plasticity.

In fact, these circuits are very plastic after SCI. To explore these networks, we applied a trans-synaptic retrotracer to the diaphragm, the main respiratory muscle, to be able to mark both the motor neurons that innervate it (phrenic motor neurons) and the interneurons implicated in these networks. We observed that phrenic motoneurons have PNN, but thinner than those seen in lumbar motoneurons, while interneurons do not have these networks, compared to neighboring interneurons (not involved in the respiratory circuits) that have very prominent ones. This lack of PNN in the interneurons would allow the circuit to be very plastic and adaptable to the needs of the moment.

3. Relevance of the results and future implications

In this project, through the use of animal models, we have studied spinal PNNs and their strategic role in maintaining an adequate balance between stability and plasticity in the mature nervous system, as well as after spinal cord injuries. We have also evaluated how physical activity can modulate these PNNs and promote functional recovery after injury. Physical rehabilitation is one of the most widely used therapies after traumatic spinal cord injuries, but the mechanisms of action that lead to its proven benefits are unclear.

In fact, it is often assumed that exercise, by promoting plasticity, helps to improve neural function. However, in our project we show that physical activity has different effects in the brain and the spinal cord, since it differentially modulates PNNs in these two anatomical regions. It is widely accepted that physical activity in the brain promotes plasticity by reducing PNNs around different neurons,. This may explain much of the positive effects of physical rehabilitation on functional recovery after spinal cord injury, as it favors the upper centers being more plastic and partially compensates for the injury. In contrast, we find that physical activity increases PNNs around spinal motoneurons, which promotes the stability of spinal circuits and limits their plasticity. This is very interesting, as the spinal cord injury itself causes a reduction in the PNN of these spinal motor neurons below the injury due to the denervation they suffer from the upper centers, which leads to less activation of these neurons. Physical activity preventing or reducing this loss of PNN limits the plasticity of the spinal circuits, therefore preventing the maladaptive plasticity that is usually observed after spinal cord injuries (spasticity, neuropathic pain etc.)

The findings are very relevant because they show that, while plasticity after an SCI can be beneficial and promote the regeneration of injured tracts or spare axons that can compensate the functional loss, it is also essential to maintain the stability of circuits to ensure their good functionality. The fact that physical activity differentially modulates PNNs in the spinal cord and the brain is very paradigmatic and reinforces the use of physical rehabilitation in spinal cord injuries, but providing evidence that this activity will not promote plasticity in the spinal circuits; on the contrary, it will guarantee stability. This stability will be positive and will limit maladaptive plasticity, but in turn can limit the regeneration of injured axons and therefore interfere with combination

therapies that seek to promote plasticity and spinal cord regeneration. Understanding the mechanisms of action of rehabilitation therapies will improve their clinical applicability and clarify their benefits but also their limitations, and will make it possible to combine therapeutic strategies more effectively.

4. Generated scientific bibliography

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