Differences in expression of perineuronal nets in cervical spinal cord across species, development and cell types

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Introduction

Perineuronal nets (PNNs) are extracellular matrix molecule aggregations that form around certain neurons in the CNS.

➢They are involved in the control of neuronal plasticity and axon regeneration.
➢Time of appearance of PNNs during development equal to the ending of critical period of plasticity.
➢Deposition of PNNs around neurons helps to stabilize the established neuronal connections and to restrict plastic changes due to future experience within CNS.
➢The study of this molecules is therefore of paramount importance and relevance to stroke research.

Research aims

➢To detect the differences in expression of PNNs in:

- different species (monkeys and rats),
- several compartments of spinal cords

➢To identify associations of PNNs and different cells types

Methods

➢Thin sections of rat and monkey cervical spinal cord were taken and then incubated with several primary antibodies (against PV, calbindin, ChAT and VAChT). PNNs were stained with a biotinylated lectin.

➢Secondary antibodies were added which bind to their respective primary antibodies, and fluorescent avidin that bound to biotinylated lectins, and fluoresced when exposed to a particular wavelength of light. (see figure below)

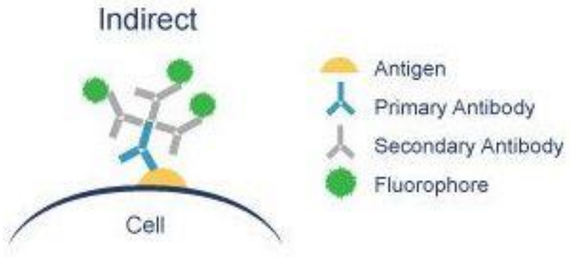

Figure 1: An illustration of how indirect fluorescence works.

➢Neurons surrounded by PNNs could be visualised using a fluorescence microscope.

Results and Discussion

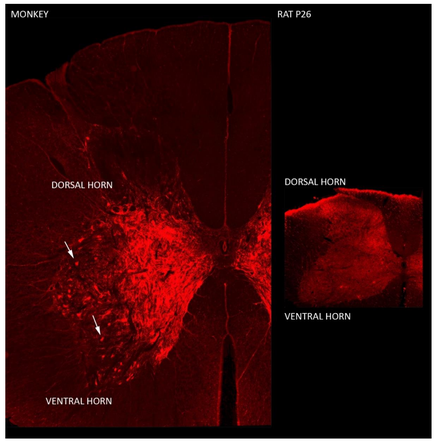

Figure 2: Lectin-stained spinal cord of monkey and rat Postnatal day (P26). PNNs are very defined in certain areas for monkey such as medial spinal cord, with lateral motorneuron pools, superficial dorsal horns and central canal having the least density of PNNs. Arrows point to auto-fluorescent motoneurons with no PNNS around them. In rat P26, PNNs are diffusely distributed throughout the spinal cord.

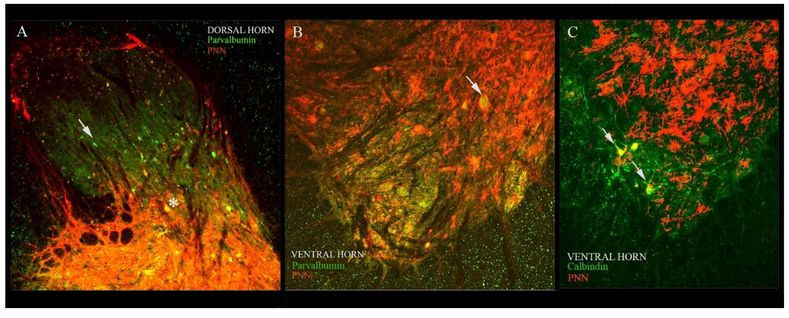

Figure 3
In cerebral cortex, PV and CB positive interneurons are usually surrounded by PNNs but it does not always appear to be the case in spinal cord.

A,B: A comparison of PV staining in dorsal and ventral horns of monkey. It is shown that there are not much PNNs around PV cells in superficial dorsal horn of monkey, possibly due to plasticity in that region because superficial dorsal horn modulates response to pain signal [1].

C: Calbindin staining in ventral horn of monkey. Renshaw cells which are interneurons that provide a regulatory feedback system to control the excitability of motoneurons [2], are stained by calbindin in monkey (arrow) and have varying proportion of expression of PNNs around them.

Results (continued)

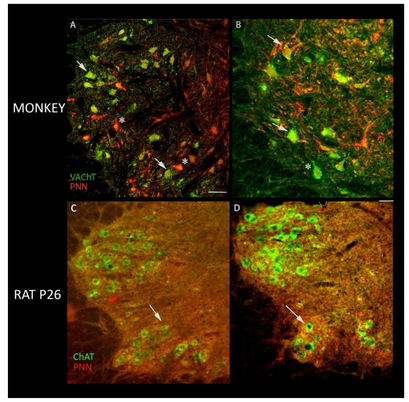

Figure 4
A,B: Lower and higher magnification image of VAChT and lectin staining of monkey showing low expression of PNNs around the alpha motorneurons which are surrounded by VAChT-positive cholinergic terminals. (arrow in A) Some nearby neurons (asterisk in B) have intense PNN, possibly being γ motor neurons or interneurons.

C,D: Lower and higher magnification image of ChAT and lectin staining of rat P26. Motor neurons, stained by ChAT are shown to have higher concentration of PNNs around them in rat compared to those in monkey.

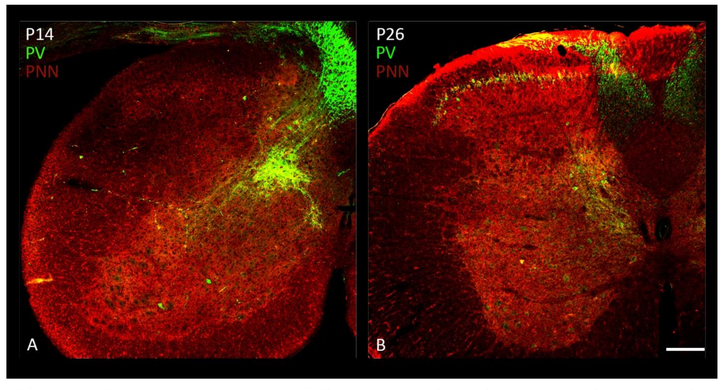

Figure 5: A comparison of PV and lectin staining between rats P26 and P14. It can be seen that PV staining changes with age and PV positive interneurons appear at P26, but PNNs are present before PV neurons.

Conclusion

This experiment demonstrates that monkey differs from rat, with a larger area in the dorsal horn without PNN expression, and in addition, no staining around motorneurons and the central canal. This indicates there could be more chance for plasticity in response to stroke, with unaffected pathways able to sprout and innervate motorneurons when the corticospinal tract is damaged. Therefore, rodent models may not be the best for this kind of basic research.

References: [1] Moore, Kimberly A., Hiroshi Baba, and Clifford J. Woolf. "Synaptic transmission and plasticity in the superficial dorsal horn." Progress in brain research 129 (2000): 63-80.
[2] Moore, Niall J., et al. "Synaptic Connectivity between Renshaw Cells and Motoneurons in the Recurrent Inhibitory Circuit of the Spinal Cord." The Journal of Neuroscience 35.40 (2015): 13673-13686.