Interspecies Comparisons of Cellular Organization in Spinal Pain Processing Network

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Background: The spinal dorsal horn (SDH) is the first site in the central nervous system where sensory information is processed before refined signals are relayed to the brain and underpin perception. 95% of the cells in this region are 'local processing' interneurons comprising inhibitory (I) and excitatory (E) neurons, while a small population of Projection Neurons (PN) transmits sensory information to the brain. Research continues to build a sophisticated understanding of these circuits, but most of this information comes from rodent studies. This leaves a large knowledge gap in the organisation of spinal dorsal horn E/I circuits across mammalian species outside mouse and rat.

Methods: Perfusion-fixed spinal cord from mouse, rat, cat, and monkey, including the lumbar enlargement, have been collected for immunolabelling analysis to characterise the conservation and organisation of E/I neuronal circuits across species. In addition, a lipophilic dye tracing approach (1,1-dioctadecyl 3,3,3′,3′-tetramethylindocarbocyanine perchlorate - DiI) has been validated to identify putative projection neurons in fixed tissue samples. Cell counts are undertaken on confocal image stacks (30 optical sections, z-steps 1 μm), including labelling combinations of all neurons (NeuN), excitatory interneurons (Lmx1b), inhibitory interneurons (Pax2), and projection neurons (DiI). Analysis uses the Image J/Imaris view software..

Results: This report presents protocol optimisation to compare E/I interneuron and PN distributions in mouse, rat, cat, and monkey. Data are presented confirming cross species immunolabelling protocols using a consistent antibody panel are established. In addition, an improved, optimized protocol to DiI-label projection neurons has been validated in mouse, cat, and monkey. This achieves selective labelling of putative projection neuron profiles in the superficial and deep dorsal horn across species

Conclusions/Discussion: The dorsal horn of the spinal cord is particularly relevant in chronic pain. Our working model of the complex circuits in this region, containing several subpopulations of inhibitory and excitatory interneurons as well as projection neurons, has been built using rodent data. This project seeks to test the assumption that the same information can be directly translated across other mammalian species and ultimately to humans.