Characterisation of the projection neurons innervated by Trpm8-expressing afferents in lamina I of the mouse spinal dorsal horn

Aimi N. B. Razlan, Raphaelle Quillet, Allen Dickie, Andrew Bell, Andrew J. Todd

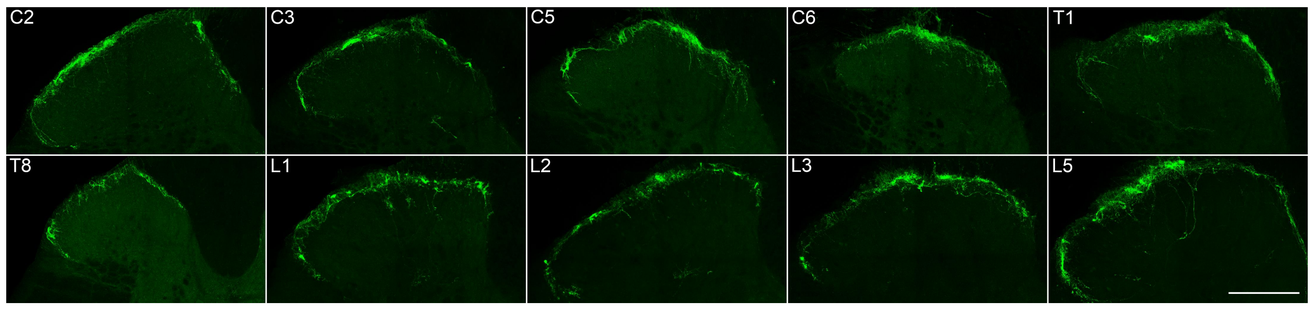
Spinal Cord Group, School of Psychology and Neuroscience, University of Glasgow

INTRODUCTION

The anterolateral system (ALS) plays a crucial role in transmitting somatosensory information, such as pain, itch and skin temperature, from the spinal cord to multiple brain areas. This information is conveyed to the brain via projection neurons, and many of these cells located in lamina I of the spinal cord. Although most lamina I ALS neurons respond to noxious stimuli, a distinct subset is selectively activated by cooling of the skin (Hachisuka et al., 2020). These neurons are activated by primary afferents expressing Trpm8, a cold-sensing ion channel. Here, we aimed to investigate the connection between Trpm8-expressing primary afferents and lamina I ALS neurons, using a Trpm8^{Flp} mouse crossed with a reporter line for Flp (RCE:FRT). Many ALS neurons transiently express the transcription factor Phox2a. By using a more complex mouse line (Phox2a::Cre;Ai9;Trpm8^{Flp};RCE:FRT), we demonstrated that Phox2a-derived lamina I projection neurons are innervated by Trpm8-positive afferents. Single-nucleus RNA sequencing identified that these densely innervated cells belong to a distinct transcriptomic class known as ALS3.

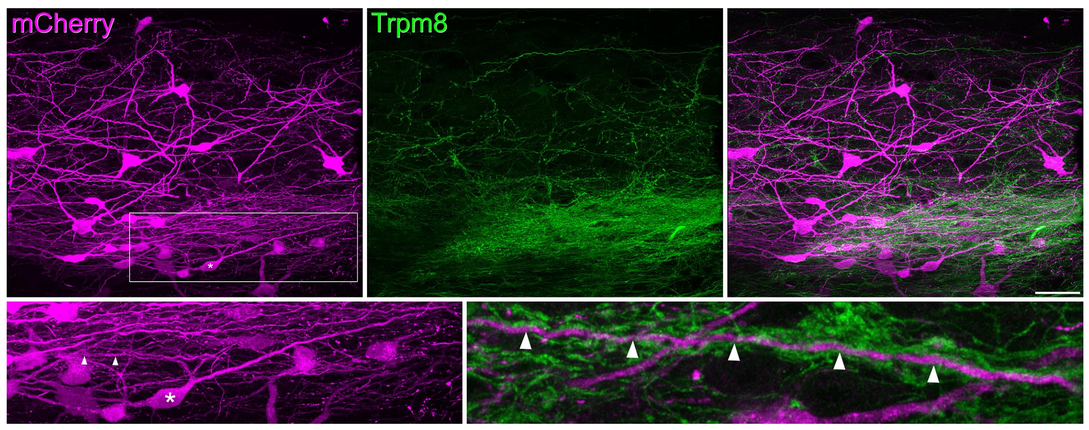
RESULTS

Distribution of Trpm8-expressing afferents in the spinal dorsal horn



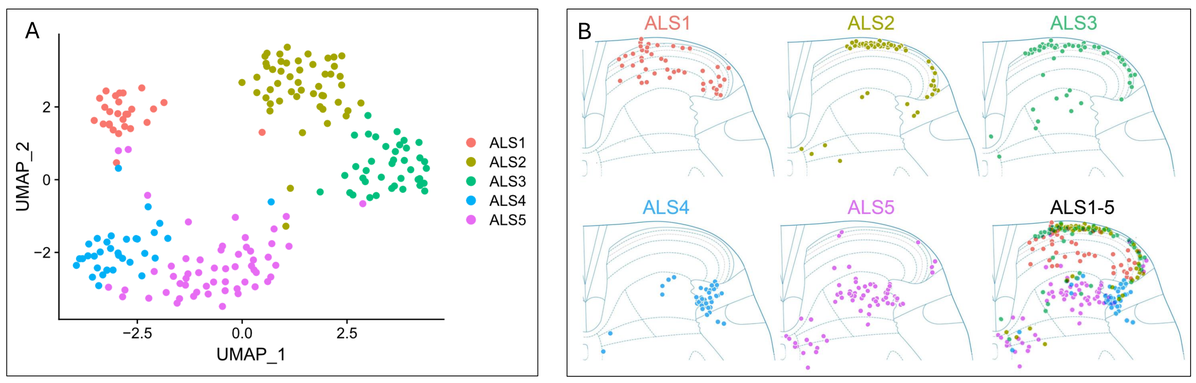
- Trpm8^{Flp};RCE:FRT mice → Trpm8-expressing afferents express green fluorescent protein (GFP)
- Trpm8 axons formed discontinuous bundles that were primarily restricted to lamina I of the spinal dorsal horn

Input fromTrpm8-expressing afferents to lamina I projection neurons



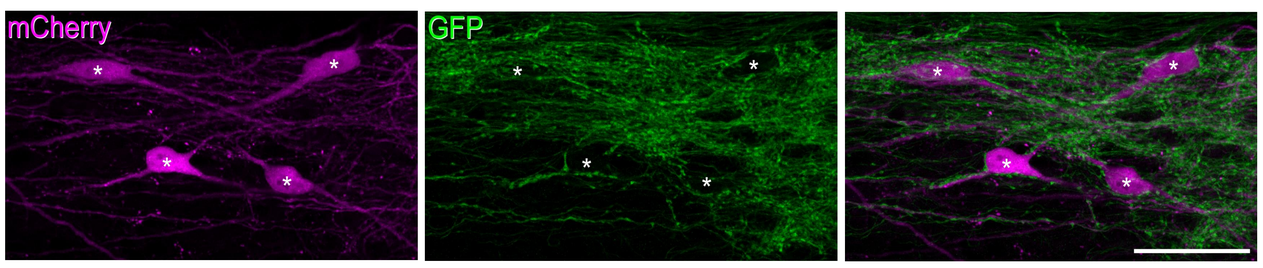
- Trpm8^{Flp};RCE:FRT mice received AAV9.mCherry injection into the lateral parabrachial (LPb) region
- Numerous retrogradely labelled projection neurons with mCherry and Trpm8-expressing afferents express GFP in lamina I
- Trpm8-expressing afferents formed a complex meshwork of bundles seen in the horizontal sections

Transcriptomic clusters detected by single-nucleus RNA sequencing among Phox2a-derived ALS neurons



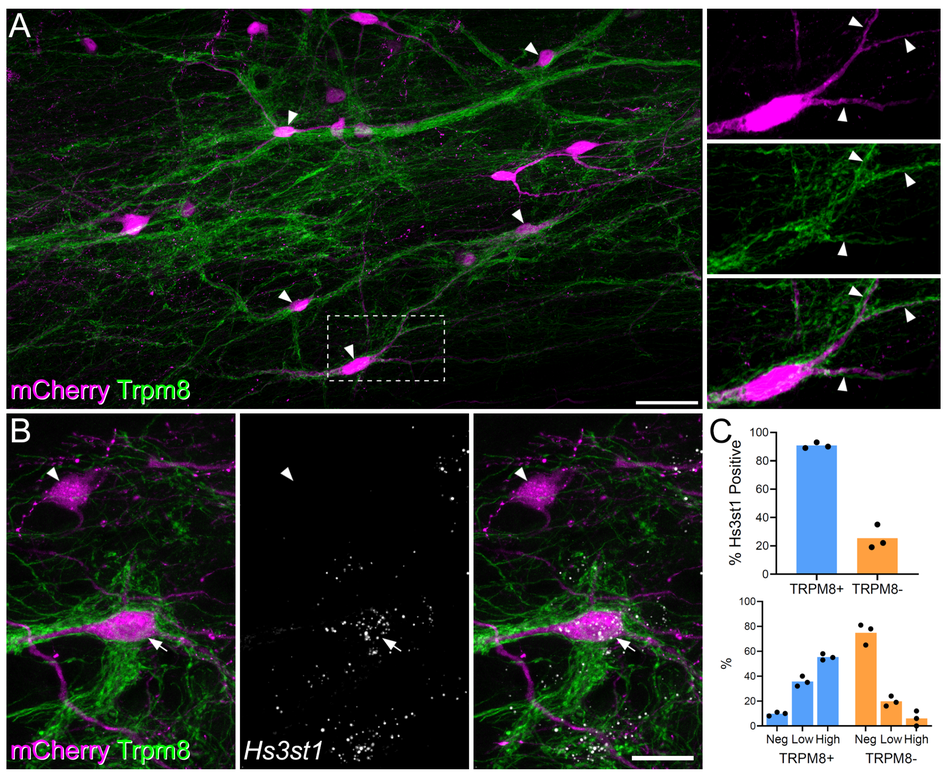
- UMAP plot showing five clusters (ALS1-5) detected within Phox2a nuclei, each defined by unique gene expression patterns
- Plots showing the laminar distribution and location of Phox2a cells in each cluster

Phox2a-lineage cells that are associated with Trpm8-expressing afferents



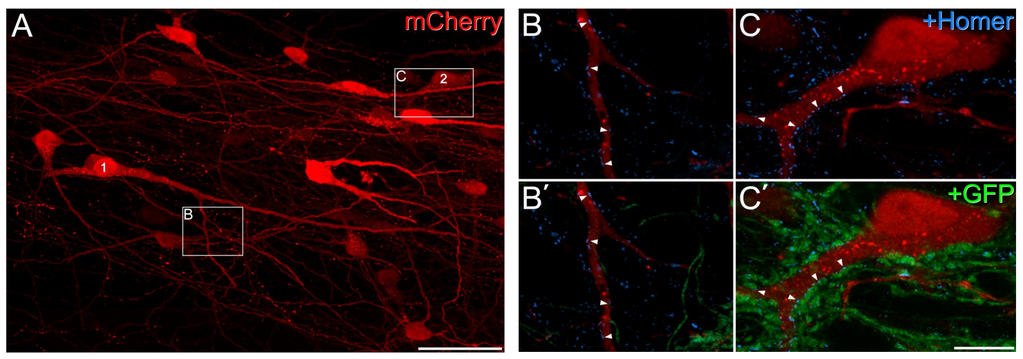
- Phox2a::Cre;Ai9;Trpm8^{Flp};RCE:FRT mice → Phox2a+ ALS neurons labelled with mCherry and Trpm8-expressing afferents with GFP
- In L4 segments, ~24% of retrogradely labelled Phox2a+ neurons were densely coated by Trpm8-expressing afferents

Projection neurons with dense Trpm8 input are likely correspond to ALS3



- Horizontal sections revealed spinoparabrachial neurons retrogradely labelled with mCherry were densely innervated by GFP-labelled Trpm8 axons
- In L4 segments, ~21% of retrogradely labelled lamina I ALS neurons had dense innervation from Trpm8-expressing afferents
- 91% of these neurons with dense Trpm8 input were positive for *Hs3st1*, a marker of ALS3 neurons
- ALS3 cluster cells preferentially receive input from Trpm8 afferents and are likely include cold-selective cells

Quantification of synaptic input to lamina I ALS neurons from Trpm8-expressing afferents



- Immunostaining for the postsynaptic density protein Homer showed that these neurons received numerous excitatory synapses from Trpm8 afferents
- In densely innervated cells, 62% of excitatory synapses originating from Trpm8 boutons, compared to only 6% in cells without dense Trpm8 innervation

CONCLUSIONS

- Trpm8-expressing afferents innervate lamina I, forming a meshwork of bundles, particularly visible in horizontal sections
- Trpm8 axons within these bundles form numerous contacts onto subset of lamina I ALS neurons
- These neurons with dense Trpm8 input are likely to correspond to ALS3 cluster identified in Bell et al., (2024)
- Phox2a+ neurons are included among those innervated by Trpm8-expressing afferents

REFERENCES

- Hachisuka et al., 2020. Selective-cold output through a distinct subset of lamina I spinoparabrachial neurons. Pain. 161:185-194
- Bell et al., 2024. Deep sequencing of Phox2a nuclei reveals five classes of anterolateral system neurons. PNAS. 121(23)