Nociceptive inputs and wake-promoting projection neurons in the Lateral Parabrachial Nucleus (lPBN)

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Background

Chronic pain is pain that has persisted beyond normal healing time and has a profound impact on quality of life. The disease affects 1 in 5 Australians and is associated with insomnia in up to 50-90% of patients (Deloitte, 2019). Understanding the association between neuronal circuits mediating pain and those that promote wakefulness is an integral step to understanding how these comorbidities develop.

We investigate:

- if the same lPBN projection neurons send inputs to the two hypothalamic-wake regions
- Then we attempt to use a trans-synaptic AAV strategy to demonstrate if hypothalamic-wake projecting neurons receive direct inputs from the SCDH.

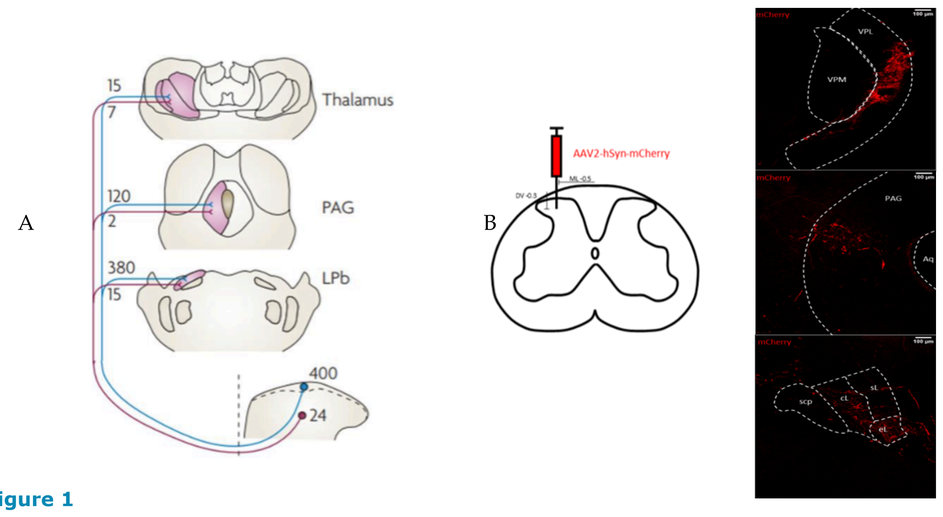
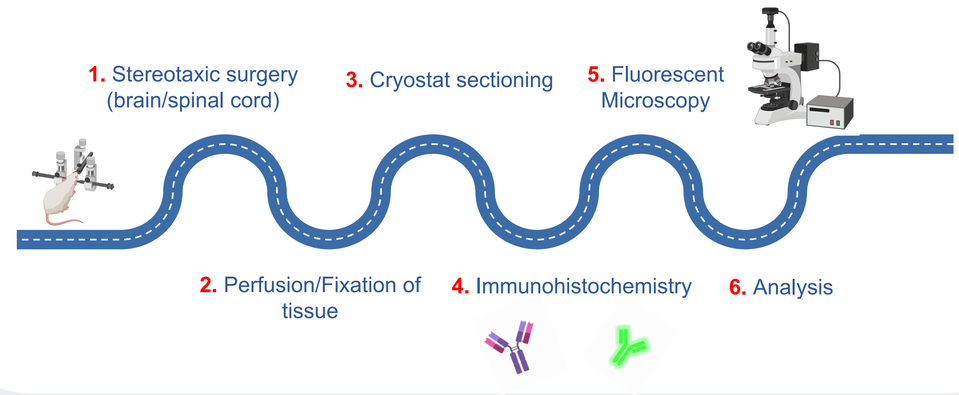


Figure 1

A. Cartoon of the Anterolateral Ascending Pain Pathway (R. Power, 2020). Nociceptive projections from the superficial lamina of the spinal cord dorsal horn (SCDH), send axons to the lateral parabrachial nucleus, periaqueductal gray (PAG) and thalamus.
B. Anterograde tracer injected into the SCDH labels projections to the lPBN, PAG and thalamus

Research Plan

- Use AAV-vectors to transfect cell populations in the spinal cord dorsal horn (SCDH), lPBN and two hypothalamic-wake regions with fluorescent tracers
- Image brain and spinal cord slices using fluorescent microscopy.



Hypothesis

Nociceptive information into the PBN is passed to neurons that project to the hypothalamic areas known to drive a form of wakefulness

Aims

To investigate if PBN neurons that receive nociceptive input from SCDH also project to LH/POA

1) Determine if the same lPBN neurons project to two hypothalamic areas (LH/POA) using a retrograde tracer and fluorescent markers
2) Control experiment to confirm that the PBN does not have descending projections to SCDH
3) Test AAV1-hSyn-Cre to see if it can trans-synaptically label neurons in the lPBN that directly receive input from the SCDH

Results

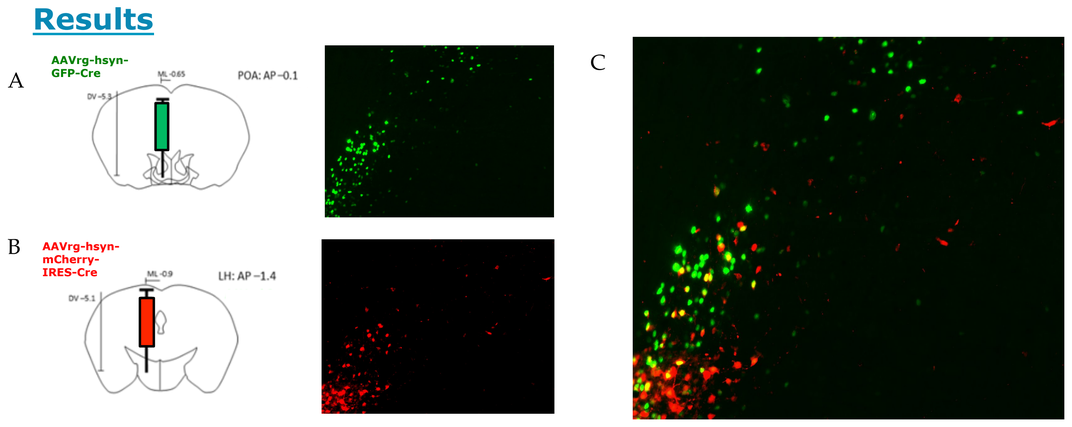
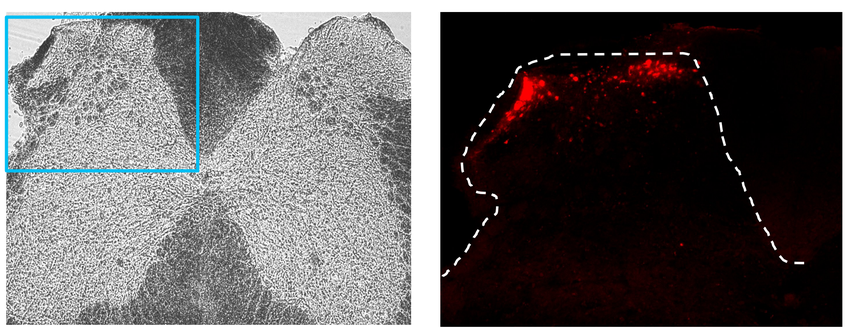


Figure 2. A. Retrograde tracer (AAVrg) injected into the pre-optic area of the hypothalamus (POA, red). B. Retrograde tracer (AAVrg) injected into the lateral hypothalamus (LH, green). C. Composite image of the lPBN showing some neurons which send collaterals to both the LH and POA.

Trans-synaptic AAV strategy

- AAV1 has both trans-synaptic and retrograde labelling ability. It is only useful if the SCDH to lPBN connection is unidirectional.
- To test AAV1 as a trans-synaptic tracer, a retrograde tracer was injected in the SCDH.

Figure 3. SCDH injection site of retrograde tracer. The retrograde tracer transfected primarily the lamina I region of the dorsal horn.



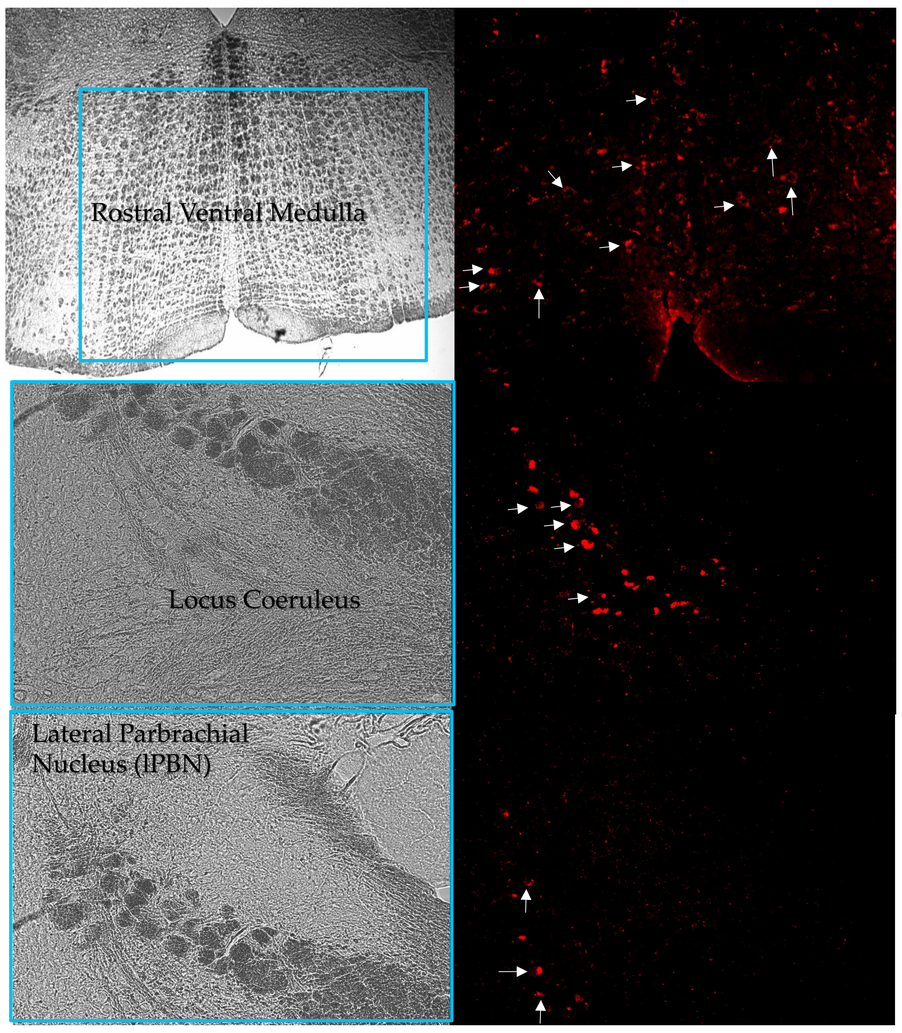


Figure 4
Expression of cell bodies in the LC, RVM but not in the lPBN from SCDH retrograde injections.

Conclusions

- A subset of lPBN projection neurons send collaterals to both hypothalamic-wake regions
- The AAVrg tracer in the spinal cord shows there are descending projection neurons in the LC and RVM but not lPBN, indicating that the SCDH to lPBN projection is suitable for trans-synaptic labelling using AAV1-hSyn-Cre
- We have injected AAV1-hSyn-Cre into the SCDH of a Cre-reporter mouse and are currently analysing the ability of this trans-synaptic marker to label the lPBN targets of SCDH projection neurons

References
Deloitte (2019). The Cost of Pain in Australia.
R. Power (2020).