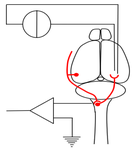
Establishment of a preclinical migraine model based on nitroglycerine-induced sensitization of spinal trigeminal parabrachial neurons in the anaesthetized rat

J. Allard[1], O.Toury[2], A.A. Asuni[3], F. Gastambide[3], B. Buisson[2] and B.J. Hall[3]
[1]E-Phys, Clermont-Ferrand, France; [2]Neuroservices-Alliance, Aix-en-Provence, France; [3]Lundbeck A/S, Valby, Danemark
Julien.allard@neuroservices-alliance.com

Aim

- To gain understanding on nitroglycerine (NTG)-mediated pain sensitization related to migraine, in addition to providing a potential platform for therapeutic screening.
- Strategy is based upon 1) electrophysiological measures of the activity of trigeminocervicoparabrachial (TPB)[1,2] neurons innervating the periorbital region and 2) the ability of NTG[3] to induce migraine-like symptoms in rodents.
- We hypothesize that spinoparabrachial neurons, which are thought to play an essential role in maladaptive pain, should play an essential role in the generation of pain-related migraine.

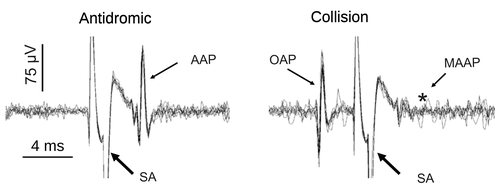
Set up and method



- Extracellular recording of lamina I and III-V TPB neurons under isoflurane anaesthesia.
- Search of TPB neurons based on antidromic stimulations from the PB area and obtention of positive collision test.
- 5 i.p. injections of NTG 10 mg/kg or vehicle (VEH) every other day.
- Electrophysiological measures performed 24-28 h after last injection.

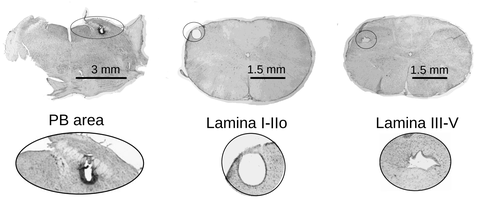
Identification of lamina I and III-V TPB neurons

Electrophysiology



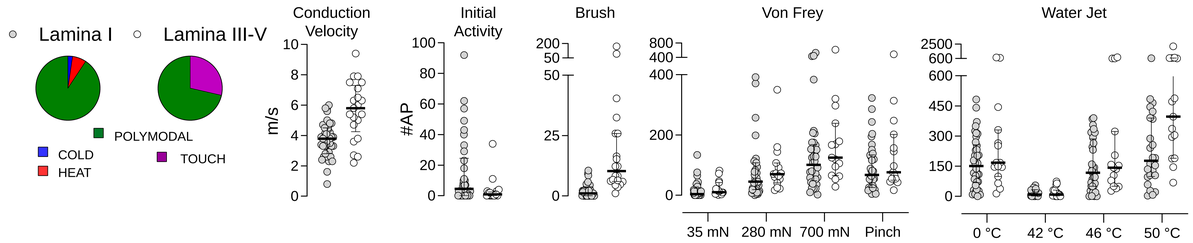
- "Antidromic" and "Collision": overlay of 8 successive responses.
- SA, stimulus artefact; AP, action potential; AAP, antidromic AP; OAP, orthodromic AP; MAAP, missing AAP.
- In collision mode, any detected spontaneous or evoked action potential triggers an antidromic stimulation from the PB area.

Histology



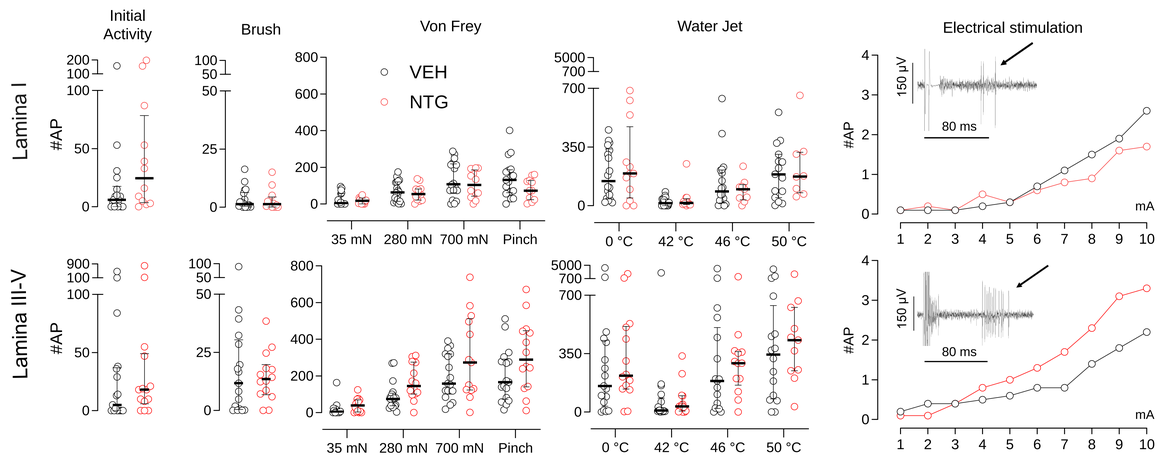
Tissue sections were generated to locate antidromic stimulation sites in the PB area (Perls' Prussian blue) and recording sites in the cervical cord (electrolytic lesion).

Experiment #1, control condition only: marked spontaneous activity in lamina I and exclusive light touch responses in lamina III-V.



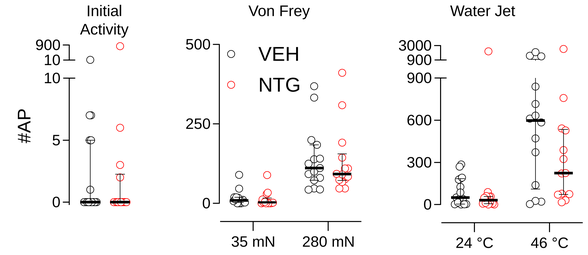
- Twenty six rats experimented, 43 and 21 TPB neurons recorded in lamina I and III-V, respectively.
- Pie charts: distribution of modalities within the population of neurons recorded.
- Conduction velocity: between antidromic stimulation site and recording site (axon length estimated to 1.5 cm).
- Responses expressed as number of action potentials (AP); initial activity measured for 60 s; brush, mean of 10 successive sweeps; Von Frey and pinch applied for 6 s, measured for 5 s; thermal stimuli applied with water jet (15 ml), measured for the entire duration of the response (3-30 s).
- Data shown as individual values and median±interquartile range.
- Below: locations of stimulation and recording sites. Only 9/18 (lamina I) and 7/12 (lamina III-V) of marked recording sites were recovered.

Experiment #2, chronic Vehicle vs NTG : trend for NTG-sensitization in lamina III-V.

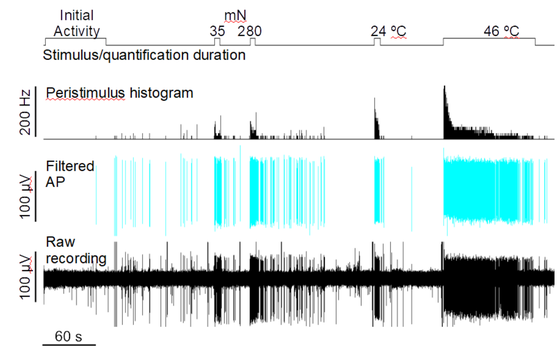


- Lamina I: 16 rats; 18 VEH and 12 NTG TPB neurons. Lamina III-V: 14 rats; 17 VEH and 14 NTG TPB neurons.
- Modality distribution and conduction velocities for lamina I and III-V TPB neurons (not shown) were similar to that observed in experiment #1.
- Electrical stimulations: 2 ms square wave pulses, 1-10 mA. Responses measured as mean number of C-fibre related action potentials (arrow on recordings).
- Statistical analysis using multiple Mann-Whitney tests and corresponding corrections did not evidence any discovery (GraphPad Prism 9.4.1).

Experiment #3, chronic NTG follow up: lack of NTG-sensitization in lamina III-V.



- A protocol focussed on lamina III-V TPB neurons using a limited number of stimuli was designed to replicate experiment #2 (example of recording below).
- Protocol was halted at intermediate read out (5 rats/group; 15 VEH and 14 NTG TPB neurons).



Conclusions

- TPB neurons innervating the peri-orbital region were found in superficial (lamina I) and deep (lamina III-V) layers of the cervical cord. Polymodal nociceptors were predominant in both regions.
- The apparent lack of sensitization of TPB neurons after chronic NTG treatment is at odd with the sensitization of trigeminal[4] and trigeminocervical[3] neurons observed after acute NTG. Sensitization of trigeminocervical neurons is thought to be the origin of the behavioural mechanical allodynia and increased c-fos immunoreactivity in the cervical cord observed after both acute[5] and chronic[6] NTG.
- In the present protocol, additional selection criteria might be necessary to unravel the sub-population of TPB neurons at the origin of NTG-induced migraine-like symptoms in rodents

1 Bester et al., 2000, J Neurophysiol, 83, 2239–2259.
2 Gauriau and Bernard, 2002, Exp Physiol, 87, 251–258.
3 Akerman et al., Brain, 2019,142, 103-119.
4 Zhang et al., Ann Neurol, 2013, 73, 741–750.
5 Tassorelli and Joseph, Brain Res, 1995, 682, 167-181.
6 Greco et al, J Headache Pain, 2018, 19, 51-59.