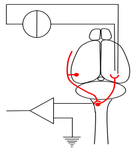
Establishment of a preclinical migraine model based on nitroglycerine-induced sensitization of spinal trigeminal parabrachial neurons in the anaesthetized rat

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.

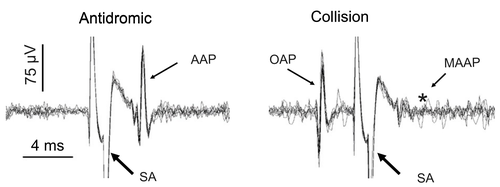
Set up and method



- 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.

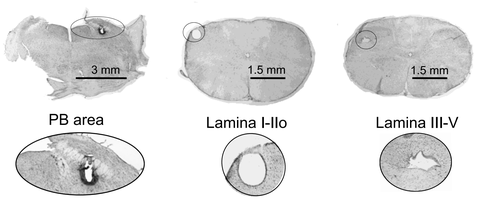
Identification of lamina I and III-V TPB neurons

Electrophysiology



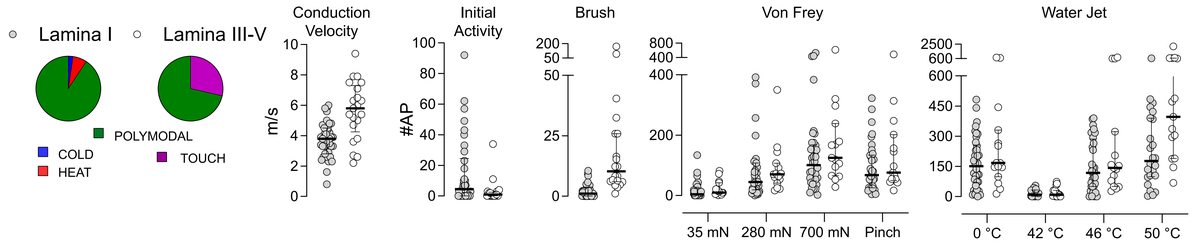
- "Antidromic" and "Collision": overlay of 8 successive responses.
- SA, stimulus artefact; AP, action potential; AAP, antidromic AP; OAP, orthodromic AP; MAAP, missing AAP.
- In collision mode, any detected spontaneous or evoked action potential triggers an antidromic stimulation from the PB area.

Histology

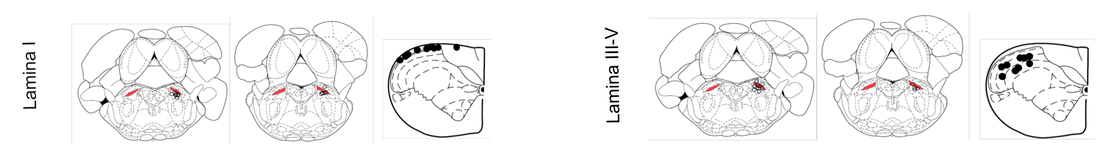


- Tissue sections were generated to locate antidromic stimulation sites in the PB area (Perls' Prussian blue) and recording sites in the cervical cord (electrolytic lesion).

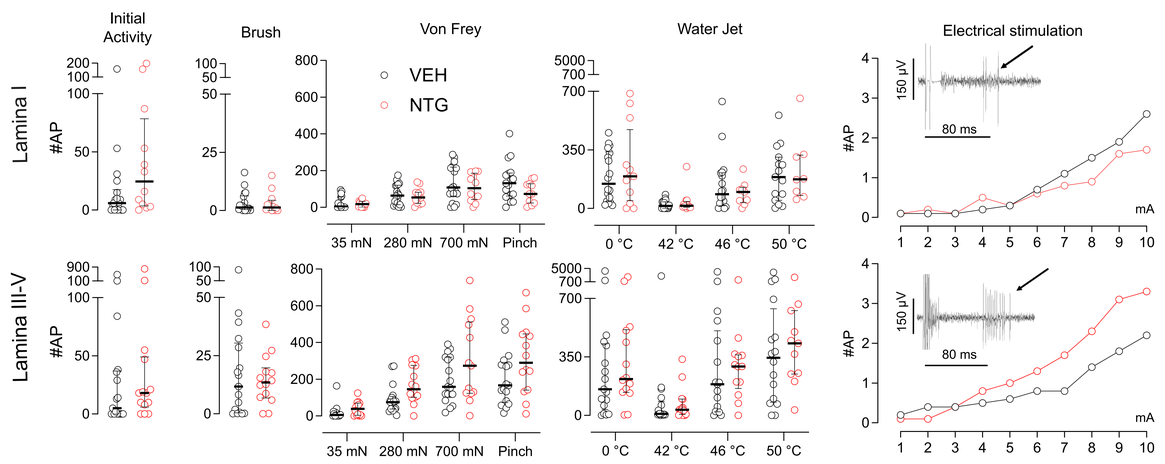
Experiment #1, control condition only: marked spontaneous activity in lamina I and exclusive light touch responses in lamina III-V.



- 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.

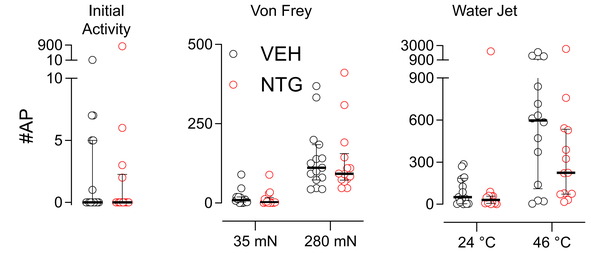


Experiment #2, chronic Vehicle vs NTG : trend for NTG-sensitization in lamina III-V.

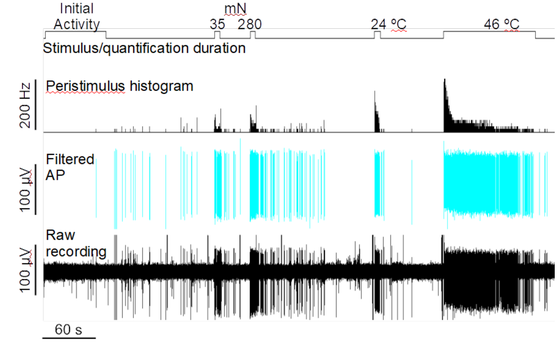


- Lamina I: 16 rats; 18 VEH and 12 NTG TPB neurons. Lamina III-V: 14 rats; 17 VEH and 14 NTG TPB neurons.
- Modality distribution and conduction velocities for lamina I and III-V TPB neurons (not shown) were similar to that observed in experiment #1.
- Electrical stimulations: 2 ms square wave pulses, 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.