D10

In vivo intracellular recording in mouse DRG neurones and identification of their sensory properties

X. Fang[1], S. Pype[2] and S.N. Lawson[1]

[1]*Physiology, University of Bristol, Bristol, UK and* [2]*CNS Discovery Research, Johnson & Johnson Pharmaceutical Research & Development, Beerse, Belgium*

Studies of dorsal root ganglion (DRG) neurons are needed for the understanding of mechanisms underlying acute and chronic pain; these neurones are ideal targets for novel analgesics. Although mice are widely used for studies of DRG neurones in pharmacological research and drug development, published electrophysiological studies on DRG neurones in this species are all *in vitro*. *In vivo* intracellular recordings allow identification of neuronal properties, e.g. sensory receptive properties and conduction velocity, within the *in vivo* environment. This is important as the *in vivo* environment influences these neurones in many ways, and can be altered considerably in chronic pain states.
We have therefore developed *in vivo* recordings in L4/L5 DRG neurones in adult mouse. Adult C57Bl6 male mice (26-35g) are deeply anaesthetized with initial sodium pentobarbitone (80-85mg/kg, i.p.) followed by regular further doses (25mg/kg, i.v.) to maintain deep anesthesia throughout. A tracheotomy allows for artificial ventilation as necessary and the left external jugular vein and carotid artery are cannulated to allow supplementary anaesthetic and blood pressure monitoring respectively. Following a laminectomy, the left L3-L6 DRGs are exposed and a pool constructed. Liquid paraffin maintained at 30°C is used to fill the pool, covering the tissues. Intracellular recordings with sharp glass microelectrodes filled with 3M KCl enable study of evoked somatic action potentials, conduction velocity measurement and identification of sensory receptive properties of DRG neurones as C, Aδ or Aα/β nociceptive neurones or as different types of low-threshold mechanoreceptive neurones. At the end of experiments, animals are killed with an anaesthetic overdose. Methods used to improve survival and recording stability will be demonstrated. Preliminary analysis of electrophysiological properties shows that the distinct patterns of somatic action potential configuration in relation to sensory properties are similar in these C57Bl6 mice to those previously reported in rats and guinea pigs.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

D12

A rapid system for applying thermal stimuli during patch clamp and $[Ca^{2+}]_i$ imaging experiments

G. Reid and D. Zorzon

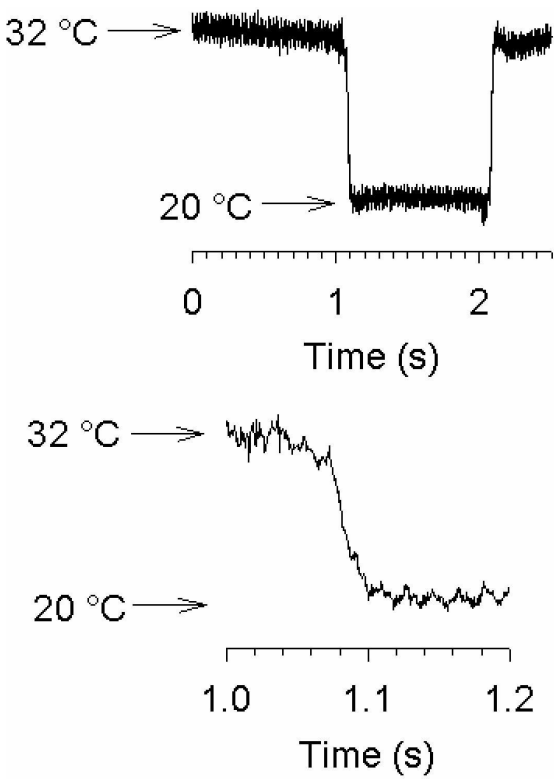
Faculty of Biology, University of Bucharest, Bucharest, Romania

Commercially available systems for temperature control during patch clamp or [Ca2+]i measurements are generally designed to maintain a constant baseline temperature. Few systems are as yet available that allow temperature itself to be used as a stimulus. Some years ago we described a system allowing heating and cooling steps with a time constant of ~5 s, or ramps up to 4 °C/s, to be applied (Reid et al., 2001). This has proven useful in our own work and that of others, but the timecourse of the stimulus nevertheless limits the experiments that can be done. This limitation led to the development of the system presented here.

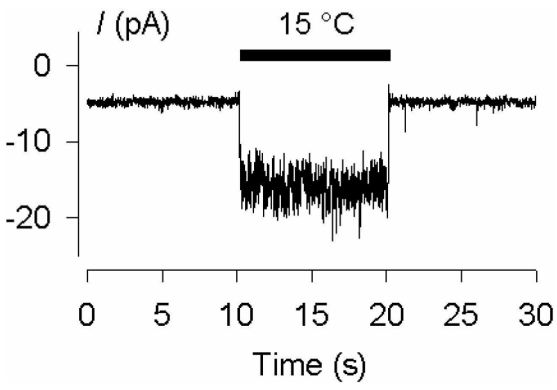
A twin version of our earlier Peltier-based system, with each Peltier unit powered by an independent feedback controller, is connected to a commercial stepper motor unit (SF-77B; Warner Instruments, Hamden, CT). Solutions flow across the Peltier elements through stainless steel tubing (18 gauge; two tubes on each Peltier element) and into 1 mm square glass outlet tubes positioned close to the cell or membrane patch being recorded. The stepper motor moves the outlet tubes so that solutions that have flowed across one or the other Peltier element can be applied to the cell or patch. This allows rapid switching between any two independently controllable temperatures, and also between control and drug solutions at the same temperature.

Switching time is typically ~20 ms between any two temperatures when 1 mm square tubing is used (Fig. 1). Theta glass allows faster switching times, but heat exchange across the thin glass septum limits the usefulness of this approach. To minimise heat exchange between the 1 mm square glass tubes we normally use, we mount the outlet tubes so that they converge at an angle of ~20° and meet only at the tip.

As well as studies on the kinetics of temperature activation of thermoTRP channels (Fig. 2), the system described here has allowed us to identify a novel group of rapidly adapting cold-sensitive DRG neurones (see communication by Babes et al., this meeting).



Time course of switching between 32 °C and 20 °C, measured using the change in offset potential at the tip of a patch clamp pipette (thermocouples and thermistors are too slow to track the fast switching). The switch from 32 °C to 20 °C is shown on an expanded timebase in the lower panel.



Rapid activation of the native cold and menthol receptor TRPM8 by a switch from 25 °C to 15 °C in a multi-channel excised patch.

Reid, G. et al. (2001) J Neurosci Methods 111:1-8

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

D13

An *in situ* preparation of the intact cerebellum: the arterially perfused hindbrain and upper body

N.L. Cerminara[1], J.A. Rawson[2] and R. Apps[1]

[1]Physiology, University of Bristol, Bristol, UK and [2]Physiology, Monash University, Melbourne, VIC, Australia

The cerebellum is the largest motor structure within the CNS, and as a result, has been the focus of intensive investigation. Studies indicate that the cerebellum is intimately involved with sensory-motor integration for the maintenance and performance of smooth and accurate movements. Many observations of cerebellar function have been obtained from work on *in vivo* animal models. For example, detailed anatomical and electrophysiological studies in anaesthetized or decerebrate animals have revealed how information from peripheral receptors in muscle, skin and joints is forwarded to the cerebellum, in addition to the 'wiring' of the mammalian cerebellum in relation to other areas of the CNS concerned with the programming and management of voluntary movement (e.g. the inferior olive, pontine nuclei, red nucleus and motor cortex). Moreover, studies in awake animals have demonstrated the modulation of cerebellar neuronal discharge patterns during the execution and coordination of movements.

In addition, the ability to manipulate the ionic environment and high mechanical stability of *in vitro* brain slice preparations has provided important details of the cellular mechanisms and synaptic events involved in cerebellar function. These preparations, however, exclude neuronal connections with other areas of the CNS as well the rest of the body. Given that many studies have demonstrated that normal cerebellar function is dependent on maintaining its connections with other brain structures (especially the inferior olive), we have therefore developed a non-pulsatile, perfused hindbrain and upper body preparation (PHBP) to study the neuronal mechanisms of the intact cerebellum *in situ*. Previously, a similar preparation (lacking a cerebellum) has been used to examine brainstem mechanisms that underlie cardio-respiratory control (Paton, 1996; Potts et al. 2000).

All surgical and experimental procedures were approved and performed in accordance with local animal welfare guidelines. In the experimental procedure Wistar rats of either sex weighing between 85-120 g (4-6 weeks of age) are deeply anaesthetized with halothane and bisected below the diaphragm. The upper body is perfused via the descending aorta with a Ringer solution gassed with 95%O_2-5%CO_2 mixture via a double lumen cannula at a pressure of 75-80 mmHg, and brain temperature is maintained at 33°C. Perfusion pressure is monitored via the second lumen of the cannula. The flow rate of the perfusate is altered until phrenic nerve activity monitored via a suction electrode displayed a eupnoeic (ramp-like) pattern.

To illustrate the viability of the cerebellum in the PHBP a number of procedures will be carried out during the demonstration: (i) Electrical stimuli will be applied to the surface of the cerebellar cortex and the brachial plexus to evoke parallel fibre and cerebellar field potentials respectively. (ii) Microstimulation of nucleus interpositus will be used to evoke EMG activity in an ipsilateral forelimb muscle (e.g. biceps brachii). And (iii) extracellular recordings will be made from single cerebellar neurones with high impedance glass-insulated tungsten microelectrodes (3-5 MΩ).

Paton JFR (1996). *J Neurosci Methods* **65**, 63-68.

Potts JT, Spyer KM & Paton JF (2000). *Brain Res Bull* **53**, 59-67.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.

D14

Modulation of Aδ- and C-nociceptor evoked responses following neuronal activation at physiologically identified sites in the midbrain periaqueductal grey

J.L. Leith, S. Koutsikou, L.F. Donaldson and B.M. Lumb

Physiology, University of Bristol, Bristol, UK

Myelinated (Aδ-fibre) and unmyelinated (C-fibre) nociceptors convey different qualities of the pain signal (first and second pain, respectively) and they are believed to play different roles in the development and maintenance of chronic pain. As such, studies that use differential activation of Aδ- and C-nociceptors should provide important insights into the central processing and modulation of nociceptive inputs.

Here we demonstrate a technique originally described by Yeomans *et al.* (1996a, b), and further modified in this laboratory (McMullan *et al.* 2004), that uses different rates of skin heating to preferentially activate either Aδ- or C-heat nociceptors. We use this approach to study the effects of descending control from the midbrain periaqueductal grey (PAG) on withdrawal and autonomic reflex responses evoked by Aδ- or C- heat nociceptors.

A male Wistar rat (280-300g) will be deeply anaesthetised using halothane (2-3% in O_2; Merial Animal Health Ltd, UK) and the right jugular vein and carotid artery cannulated. Following preparatory surgery, anaesthesia will be maintained by continuous intravenous infusion of sodium pentobarbital (31mg kg^{-1} h^{-1}; Sigma, UK). The animal will be placed in a stereotaxic frame and a small craniotomy performed.

Electromyographic activity (EMG) will be recorded from the biceps femoris via an intramuscular bipolar electrode (two Teflon-coated stainless steel wires, 0.075mm in diameter; Advent Research Materials, UK). EMG will be used to monitor the thresholds and magnitudes of withdrawal responses to the different rates of skin heating. Arterial blood pressure recorded from the carotid artery will be used to monitor effects of peripheral and central stimulation on cardiovascular responses.

A T-type thermocouple will be used to monitor skin surface temperature at the centre of a copper plate positioned at the focus of a radiant heat source. Fast (7.5°C s^{-1}) or slow (2.5°C s^{-1}) rates of skin heating will be applied to the dorsal surface of the hind paw to preferentially activate Aδ- or C-heat nociceptors, respectively. These rates have been shown previously to produce linear subsurface increases of 2.5 and 0.6°C s^{-1}, respectively. Output from the thermocouple, the filtered and amplified EMG, and blood pressure signals will be digitised and displayed on a PC running Spike2 software (CED, UK).

A glass micropipette (Harvard Apparatus Ltd, UK), containing 50mM D,L-homocysteic acid (DLH; Sigma, UK), will be driven vertically into the brain to a depth of between 4.25 and 5.7mm below the cortical surface, at approximately 7.6mm caudal to bregma and 0.9mm lateral to the midline in order to reach the dorsolateral or ventrolateral PAG. The pipette will be connected to a 1μl glass syringe (SGE, Australia) in order to administer 30nl microinjections of DLH .

Cardiovascular and reflex withdrawal responses to fast or slow rates of skin heating will be compared before and after DLH-evoked neuronal activation in the PAG in order to determine the extent to which descending control modulates reflex responses to Aδ- and C-nociceptive inputs.

McMullan S, Simpson DA & Lumb BM (2004). J Neurosci Methods 138, 133-139.

Yeomans DC & Proudfit HK (1996a). Pain 68, 141-150.

Yeomans DC, Pirec V & Proudfit HK (1996b). Pain 68, 133-140.

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Where applicable, the experiments described here conform with Physiological Society ethical requirements.