Absence of a change in amplification constant of transduction in human rod photoreceptors following bright illumination

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In rat rods it has recently been shown that prolonged exposure to moderately bright illumination causes a massive relocation of the G-protein transducin from the outer to inner segment of the cell, and a reduction of ~5× in the amplification constant of transduction measured from the *a*-wave of the electroretinogram (ERG) (Sokolov *et al.* 2002). We have now looked for a similar change of amplification constant in human rod photoreceptors.

We recorded the *a*-wave of the ERG in response to ganzfeld illumination (Thomas & Lamb, 1999), in two subjects (T.D.L. and E.N.P.). Ethical approval was given by the Cambridge Human Biology Research Ethics Committee. We first recorded responses to a family of flashes under dark-adapted conditions and then, after exposure of the eye to bright steady illumination for 30 min, we measured the recovery of responsiveness to a repeated series of dim flashes, interspersed at 2 min intervals with bright flashes. The adapting light had an intensity of 1700 cd m^{-2}, and with the dilated pupil diameter of ~7 mm gave a retinal illuminance of ~67 000 scotopic trolands, which would have bleached around 75 % of the rhodopsin.

For subject T.D.L., the normalized recoveries after one such exposure are plotted in Fig. 1, for bright flashes (○) and dim flashes (■). If the amplification constant were reduced following the exposure, then the filled symbols would be expected to be lower than the open symbols. Although a small effect is apparent at 20–30 min after the exposure, the change is only ~20 % (rather than the ~5× expected by analogy with the rat recordings), and might be explained by incomplete regeneration of visual pigment. Results for the second subject were similar.

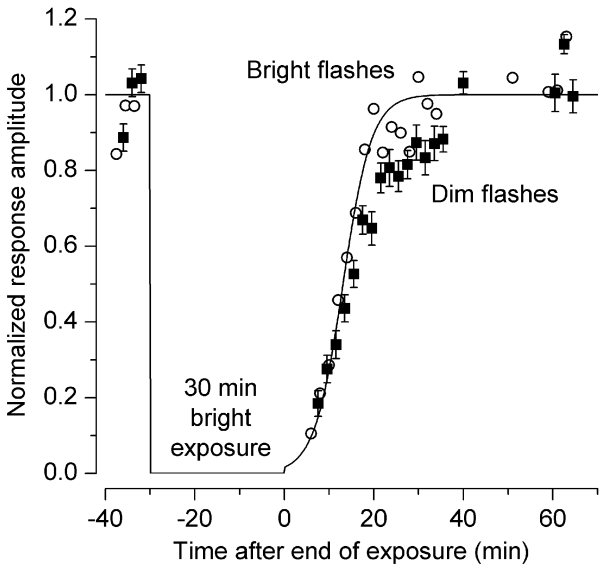

Figure 1. Normalized recoveries for bright flashes (○) and dim flashes (■) following a 30 min exposure to bright steady light. Corneal and retinal intensity, measurement time, and dark-adapted amplitude were, for bright flashes: 700 cd m^{-2} s, 27 000 troland s, 5 ms, 125 μV; dim: 3 cd m^{-2} s, 120 troland s, 15 ms, 45 μV; ± S.E.M., n = 12 flashes at 5 s intervals.

A limitation of these experiments is that the rod current is eliminated for 5 min after an exposure of this intensity, and the amplification constant cannot reliably be determined during the first 10 min or so. Our interpretation of the results is that for these observers any reduction in amplification constant following a 30 min exposure to bright light is either very small or else recovers almost completely within the first 10 min of darkness.

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All procedures accord with current local guidelines.

Substantia gelatinosa (LII) neurones in the lumbar spinal cord are inhibited by NPY in both intact and sciatic nerve-sectioned rats: an *in vitro* electrophysiological study

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In adult rats very few dorsal root ganglion neurones (DRG) express NPY; however, following injury to their peripherally projecting axons many large DRG neurones start to synthesise this peptide. In the spinal cord increased expression of NPY is seen in dorsal horn LI–LIII in the somatotopic region corresponding to an injured nerve. In these regions abnormal myelinated afferent sprouts have been observed. The present study has examined whether this new expression of NPY results in altered function of LII neurones.

Unilateral sciatic nerve section was carried out in rats between 12 and 14 days postnatally under aseptic conditions and short duration halothane anaesthesia. Sham-operated litter mates acted as controls. Seven to fifteen days later they were anaesthetised with ketamine (200 mg kg^{-1} I.M.) and decapitated. Parasaggital lumbar spinal cord slices were prepared with intact L4, L5, L6 dorsal roots, DRG and peripheral nerves, and intracellular recordings made from LII neurones. The effects of bath-applied NPY and nerve stimulation were examined. Neurones were labelled by intracellular injection of neurobiotin and subsequently slices were stained with IB4 lectin to reveal the relationship of the injected neurones to the denervated zone and LII.

Bath application of NPY (100 nM–10 μM) caused mainly hyperpolarisation of LI–LIII neurones. In the denervated zone, nine (64 %) of the intrinsic LII neurones tested with 1 μM NPY were hyperpolarised (mean amplitude −8.14 mV ± 4.18, S.D.), whereas in sham-operated animals and non-denervated regions, five (54 %) of these were hyperpolarized (mean amplitude −10.6 mV ± 4.82, S.D.) (n.s., $P > 0.5$, unpaired t test). In a sample of 39 neurones from LII–LIII (based on electrode targeting, not intracellular labelling) studied in neonatal animals not subject to prior operation, 28 (72 %) were hyperpolarized by NPY (mean amplitude −6.4 mV). Intrinsic LII neurones in either the denervated area or innervated regions were not depolarised by NPY. Immunocytochemical studies revealed that LIII–IV neurones and their dendrites projecting into LII are probably a target for myelinated primary afferents, which synthesise NPY following nerve injury. The distribution of the NPY Y1 receptor shown by immunochemistry corresponded to neurones inhibited by NPY and showed no change following nerve section. The responses to nerve stimulation suggested that no new myelinated afferent sprouts had grown following nerve section which targeted intrinsic LII neurones.

These studies suggest that the changes that occur in myelinated primary afferents following nerve injury do not result in NPY-mediated excitation of LII neurones via aberrant myelinated afferent sprouts.

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All procedures accord with current UK legislation.

Involvement of L-type calcium channels in temporal summation of a spinal reflex in the decerebrated rabbit

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In decerebrated rabbits, electrical stimulation of the sural nerve at 1 Hz and intensities of ⩾ 5 times threshold (T) activates reflexes in the ipsilateral medial gastrocnemius (MG) muscle nerve that show marked temporal summation. When sural C-fibres are activated, the summation shows differential sensitivity to blockade of glutamate *N*-methyl-D-aspartate (NMDA) receptors, such that summation of Aδ- and C-fibre-evoked reflexes is reduced, but that seen in Aβ-evoked reflexes is not (Clarke *et al.* 2002). In some preparations, spinal cord neurones show an intrinsic ability to give increasing responses to fixed stimuli that is mediated via L-type calcium channels (Russo & Hounsgaard, 1996). We have investigated the possibility that these channels might be responsible for the non-NMDA-mediated components of temporal summation in the sural-MG reflex.

Experiments were performed in ten rabbits decerebrated under nitrous oxide/halothane (2–4 %) anaesthesia. The sural nerve was electrically stimulated with 0.2 ms pulses at strengths of 5.3 T (maximal for the activation of Aβ axons), 15 T (maximal for Aδ afferents), and 92 T (maximal for C fibres). Stimulus cycles consisted of three trains of eight stimuli at 1 Hz, applied successively at Aβ, Aδ and C strengths, and were repeated once per 20 min. Animals were treated intrathecally (I.TH.) with the selective L-type calcium channel antagonist methoxyverapamil at cumulative doses of 0.03, 0.3 and 1 mg, allowing 40 min between each dose. Responses to the final control cycle and the first cycle after each drug dose were analysed for temporal summation, which was quantified by linear regression. Reflex responses were recorded from the ipsilateral MG nerve and integrated in three time bands: 5–12 ms (phase 1), 12–100 ms (phase 2) and 100–250 ms (phase 3). Experiments were terminated by intravenous injection of KCl.

In the control state with C-fibre strength stimulation, the slopes (± S.E.M.) of regression lines of reflex size against stimulus number were 40 ± 9, 124 ± 33 and 148 ± 29 μV ms stimulus^{-1} for phase 1, 2 and 3 reflexes, respectively. Methoxyverapamil (0.3 mg) significantly (*t* test, $P < 0.05$) reduced these values to 17 ± 5, 40 ± 5 and 43 ± 14 μV ms stimulus^{-1}, respectively, without having any effect on the size of the responses to the first stimulus in each train (paired *t* test, $P > 0.2$). Similar effects were seen when Aδ strength stimuli were used to activate reflexes. The data are thus consistent with the involvement of L-type calcium channels in mediating temporal summation of MG responses to sural nerve input.

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All procedures accord with UK legislation.

Nerve growth factor sensitises the cold- and menthol-activated current in thermoreceptive neurones

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Cold transduction in peripheral thermoreceptors involves a cold- and menthol-activated cation channel (Reid & Flonta, 2001), one of the TRP family (McKemy *et al.* 2002; Peier *et al.* 2002). Neurones expressing this channel depend for survival on TrkA, the nerve growth factor (NGF) receptor (Peier *et al.* 2002). Here we have tested whether NGF also alters the channel's properties.

Dorsal root ganglion (DRG) neurones were cultured from adult rats (killed by CO_2 inhalation and decapitation). After 2–4 days, cells were loaded with the Ca^{2+}-sensitive dye Calcium Green-1 AM, adapted at 32 °C and imaged during cooling ramps from 37 to 18 °C; whole-cell recordings were made using the amphotericin perforated patch configuration.

The Ca^{2+}-induced fluorescence increase on cooling (ΔF) as a fraction of basal fluorescence (F_0) was larger in cold-sensitive neurones cultured with 50 ng ml^{-1} NGF 7S (40 ± 16 %, mean ± S.D., $n = 205$) than without NGF (34 ± 16 %, $n = 48$; $P = 0.03$, Student's unpaired *t* test) and the threshold temperature was also higher (31.4 ± 3.8 °C, $n = 56$ *vs.* 28.1 ± 3.4 °C, $n = 48$; $P < 0.001$). This threshold difference was accentuated by 100 μM (−)-menthol (NGF, 35.4 ± 4.0 °C, $n = 26$; no NGF, 29.9 ± 8.0 °C, $n = 12$; $P = 0.008$). The cold-induced depolarisation was larger in neurones cultured with NGF (30.5 ± 8.9 mV, $n = 30$ *vs.* 20.4 ± 8.3 mV, $n = 16$; $P < 0.001$) and its threshold was higher (33.6 ± 2.9 °C, $n = 29$ *vs.* 28.3 ± 3.8 °C, $n = 13$; $P < 0.001$).

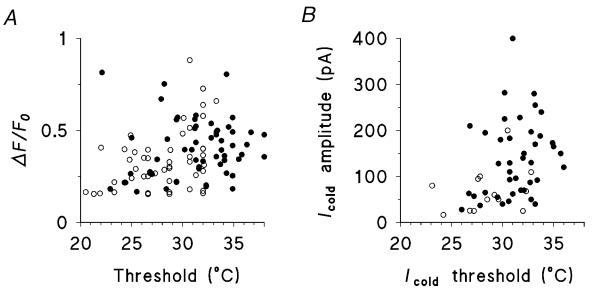

Figure 1. Amplitudes and thresholds of cold-induced increase in Calcium Green fluorescence (*A*) and cold-activated current (*B*). ●, neurones cultured with NGF; ○, without NGF.

In neurones cultured with NGF, cooling activated an inward current with a threshold of 31.3 ± 2.4 °C ($n = 42$) and an amplitude at 18 °C of 139 ± 83 pA ($n = 41$); without NGF, the current activated at 28.6 ± 2.9 °C ($n = 13$; $P = 0.002$) and its amplitude was halved (70 ± 50 pA, $n = 13$; $P = 0.007$). NGF did not change the proportion of cold-sensitive neurones (NGF, 67/923, 7.3 %; no NGF, 21/297, 7.1 %).

We conclude that NGF increases the amplitude and temperature sensitivity of the cold- and menthol-activated current in DRG neurones that already express it. As well as suggesting

intracellular mechanisms that may modulate the channel, this indicates that the NGF-induced sensitisation of nociception in conditions like inflammation is likely to be accompanied by an increase in cold sensitivity.

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All procedures accord with current local guidelines.

Intracisternal capsaicin inhibits jaw depressor and hindlimb flexor reflexes in the anaesthetized rabbit

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The trigeminally mediated jaw-depressor reflex (JDR) may be a suitable model system in which to study central actions of anti-migraine agents (Jenkins *et al.* 2000). The present study was designed to investigate interactions between dural afferents and the JDR, which involve different divisions of the trigeminal nerve, by studying effects upon the reflex of intracisternal administration of capsaicin (Cutrer *et al.* 1995). For comparison, a hindlimb flexion withdrawal reflex (FWR) has also been recorded.

Experiments were performed in twelve rabbits anaesthetized with pentobarbitone sodium (initial dose 44 mg kg^{-1}, maintenance infusion 14 mg kg^{-1} h^{-1}). The JDR was evoked by electrical stimulation of the tongue and recorded from the left digastric muscle, while the FWR was elicited by electrical stimulation of the toes and recorded from the left tibialis anterior (TA) muscle. Reflexes were evoked by 1 ms stimuli delivered in blocks of 8 at 1 Hz, and were quantified from averaged, full-wave rectified EMG signals. Capsaicin was dissolved in 15 μl DMSO and delivered to the cisterna magna in doses of 15 ($n = 6$) or 150 μg ($n = 6$). Five animals received DMSO alone prior to administration of capsaicin. Experiments were terminated by overdose of pentobarbitone followed by I.V. injection of saturated KCl solution.

Intracisternal DMSO had no significant effect on either the JDR or FWR (Friedman's ANOVA, $P > 0.3$). Given at 15 μg, capsaicin significantly (Friedman's, $P < 0.0001$) inhibited both the JDR and the FWR to medians of 10 (interquartile range (IQR) 6–26 %) and 17 % (IQR 11–27 %) of pre-drug controls, respectively. In both cases only the first time point post-capsaicin (1–3 min) was significantly different from control (Dunnett's post test, $P < 0.05$). Capsaicin (150 μg) also significantly (Friedman's, $P < 0.0001$) inhibited both reflexes (median maximum inhibition 7 and 46 % of pre-drug controls for JDR and FWR, respectively), but in this case responses were significantly different from controls for 13–15 min after the stimulus (Dunnett's, $P < 0.05$).

Thus intracisternal capsaicin inhibited both the JDR and FWR, with the higher dose inducing a longer-lasting effect. Suppression of the spinally mediated FWR indicates that the inhibition is not due to non-specific dysfunction of brainstem transmission. Our interpretation of these results is that stimulation of dural afferents (presumably C-fibres) by capsaicin activates diffuse noxious inhibitory control systems (Falinower *et al.* 1994) that impinge on neurones contributing to the JDR and FWR.

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All procedures accord with UK legislation.

Hyperalgesia to noxious heat and pinch stimuli in response to an immune challenge in the anaesthetised rat

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We recently reported a model in which to study the effects of immune challenge on nociceptive processing in the anaesthetised rat (Bleasdale *et al.* 2001). Using this model, we have now compared effects on withdrawal responses to a noxious pinch stimulus (to co-activate Aδ- and C-nociceptors) with those on withdrawal to slow rates of skin heating (to preferentially activate C-nociceptors; Yeomans & Proudfit, 1996).

Five alphaxolone/alphadalone-anaesthetised (Saffan, 14–24 mg kg^{-1} h^{-1}; I.V.) rats (275–300 g) were instrumented to record arterial blood pressure and rectal temperature. 1.2–2.0 N pinch (15 s) of the toes, and 'slow' (2.5 °C s^{-1}) ramps (30–55 °C) of contact heat of the dorsal surface of the same hindpaw were alternated once every 5 min throughout the experiment (up to 8 h) and EMG was recorded from the biceps femoris to monitor thresholds and magnitudes (modulus) of withdrawal responses. Once baseline responses were established, lipopolysaccharide (LPS) was injected (0.2 mg kg^{-1}; I.P.) to evoke an immune response and the time course of fever monitored by noting changes in core temperature every 5 min. Animals were killed with an overdose of anaesthetic (pentobarbitone, I.V.) at the end of each experiment. All values are expressed as means ± S.E.M. and an unpaired t test was used to test levels of significance.

Following a delay of 20–160 min, injection of LPS induced increases in core temperature that persisted for 95–240 min. Peak temperatures (38.0 ± 0.04 °C) were significantly increased (unpaired t test, $P < 0.0001$) from control (37.5 ± 0.04 °C). During 30 min at the peak of the fever, magnitude of EMG responses to pinch stimuli of constant forces increased significantly (unpaired t test, $P < 0.001$) by 34.5 ± 9.68 % compared with control values taken before injection of LPS. During the same 30 min period, threshold temperatures that evoked withdrawal in response to the ramped heat stimuli were lowered significantly to 49.7 ± 0.32 °C (unpaired t test, $P < 0.005$) compared with control values of 52.7 ± 0.35 °C.

This study has demonstrated hyperalgesia to heat and to mechanical noxious stimuli in response to an immune challenge. As such, these data indicate that facilitatory effects on C-nociceptor-evoked activity may constitute part of a sickness response. Any effects on Aδ-nociceptor-evoked responses remain unclear and are the subject of ongoing experiments in this laboratory.

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D.A.A.S. is a BBSRC Scholar. We thank Simon Lishman and Dave Gee for expert technical assistance.

All procedures accord with UK legislation.

Increased reliability in a modified spinal nerve injury rat model of neuropathic pain involving L5 transection plus L4 loose ligation

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For studies of the contribution of primary afferent neurones to neuropathic pain states, an animal model that shows reproducible signs of neuropathic pain, namely mechanical allodynia, thermal hyperalgesia and spontaneous pain, is required. A commonly used model of spinal nerve injury involves tight ligation or cut of the entire L5 spinal nerve (SN) close to the dorsal root ganglion (DRG) (developed from that of Kim & Chung, 1992). However, resulting measures of pain-related behaviour are variable. Lodge *et al.* (1999) suggested that this might be due to (uncontrolled) damage to the L4 SN, which runs very close to the L5 SN. We have therefore tested whether a controlled injury to L4, in addition to L5 ligation and cut, leads to reduced variability and greater effectiveness of the model.

Under sodium pentobarbitone anaesthesia (40 mg kg^{-1}, I.P.) one of four surgical procedures was carried out on day 0 on female Wistar rats (140–150 g). (1) L5 + L4, $n = 9$: the left L5 SN was tightly ligated (silk suture 6-0, Ethicon) and cut proximal to its junction with L4 SN. A chromic cat-gut (5-0, Ethicon or Look®) loop was then placed around the left L4 SN with a loop diameter at least 2 mm greater than that of the nerve. (2) L4, $n = 8$: the left L4 SN had a loose ligation as above but the left L5 SN was intact. (3) L5, $n = 8$: the left L5 SN was ligated and cut as described above. L4 SN was not ligated. (4) Sham, $n = 8$: the left L4 and L5 SNs were exposed but otherwise left intact. Behavioural tests were performed from day −3 pre-surgery (pre) to day 7 post-surgery (post). All experimental protocols were carried out under licence according to the UK Animals (Scientific Procedures) Act of 1986.

The L4 and sham groups showed no significant changes between ipsilateral and contralateral hindpaw in all tests. In both L5 + L4 and L5 groups, in all tests, the ipsilateral hindpaw became more sensitive (Friedman test). Means from pre to 7 days post for L5 and L5 + L4 groups, respectively, were as follows: mechanical allodynia (threshold to normally innocuous von Frey hairs) L5: 19.8 g (pre) to 6.9 g (post), $P < 0.05$; L5 + L4: 23.2 to 1.7g, $P < 0.001$; thermal hyperalgesia (withdrawal latency to noxious heat) L5: 16.5 to 9.8 s, $P < 0.01$; L5 + L4: 15.6 to 6.4 s, $P < 0.001$ and spontaneous pain-like behaviour (spontaneous foot liftings in a 5 min period): L5: 0 to 0.8 s, $P > 0.05$; L5 + L4: 0 to 9.7 s, $P < 0.01$. Thus for L5 + L4 the changes were larger, also the variability was smaller than in the L5 only group in all tests.

All animals were humanely killed at the end of the experiemnts.

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All procedures accord with current UK legislation.

Cytoarchitectonic organization of the functionally defined areas V6 and V6A in the parieto-occipital cortex of macaque brain

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Two areas located in the anterior bank of parieto-occipital sulcus (APOs), in the caudalmost part of the superior parietal lobule, have been recently defined on a functional basis: a ventral one, area V6 and a dorsal one, area V6A. Area V6 is a retinotopically organized purely visual area, whereas V6A contains non-topographically organized visual neurones and neurones sensitive to eye/arm movements, even in darkness.

The aim of this work was to define the cytoarchitectonic organization of APOs to look for possible anatomical evidence to support its functional subdivision in V6 and V6A. Experimental protocols complied with National law on the care and use of laboratory animals. The animals were killed with an overdose of Penthotal I.V. Architectonic analysis of Nissl material showed two main structural patterns: the 'occipital' one, located on the fundus of parieto-occipital sulcus and on the most ventral third of APOs, as rostral extension of extrastriate areas; the 'parietal' pattern, located on the dorsal two-thirds of APOs, as caudal part of superior parietal lobule. The 'occipital' cortex is characterized by a thin cortical width and a large homogeneous layer IV with densely packed granular cells; the layer III is thin, uniform and densely packed, the layer V is poorly developed and the layer VI is well defined. The 'parietal' cortex is characterized by an increase in thickness and a decrease in cellular density; the layer IV is subdiveded in two sublayers, a lower one with higher and an upper one with lower cellular density; layer III presents an increasing gradient in density and cellular size, layer V is thick and layer VI poorly defined. Two different cytoarchitectonic sectors can be further identified within the 'parietal' cortex of APOs. The ventral sector is characterized by a dense layer III and a darkly stained layer V, populated by large pyramidal cells. In contrast, the dorsal sector shows a less dense layer III and a layer V in which only isolated groups of clear pyramids are present.

The architectonic limits of all the above-described cortical subdivisions are in agreement with the electrophysiological data recorded in this region. Thus the 'occipital' cortex corresponds to the functionally defined area V6, whereas the 'parietal' cortex of the APOs corresponds to the functionally defined area V6A. The two architectonic sectors observed in area V6A indicate the presence of two subregions within this area ('V6Av' and 'V6Ad'), in agreement with the observed different distribution of functional properties and anatomical connections.

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All procedures accord with current National guidelines.

An advantageous model system for studying olfactory learning and memory

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Insect olfactory systems exhibit peculiar similarities with those of vertebrates, and offer unique advantages for studies on learning and memory formation and storage (Waddell & Quinn, 2001). Besides eliciting direct behavioural responses, olfactory input induces cardiac responses which sensitively indicate insect reactivity to odours (Angioy *et al.* 1998).

By using insect cardiac responsiveness in testing odour detection, we have designed an experimental protocol for an *in vivo* analysis of the temporal dynamics of olfactory learning and memory in flies.

Simultaneous electrophysiological recordings of cardiac activity by ECG and olfactory response from antennal sensilla stimulated with a concentration series of 1-hexanol vapours were performed on intact, restrained adult *Protophormia terraenovae* blowflies (Angioy *et al.* 1987; Angioy & Pietra, 1995). The cardiac response threshold in single specimens of a group of fifty was firstly measured as corresponding to the minimum odour concentration needed for inducing a cardiac response. Thereafter, a series of consecutive stimulations (one per minute) was performed on each fly by adopting the odour concentration corresponding to the cardiac response threshold value detected in that specimen. Effects of a series of three and of a series of six stimulations were tested on separate groups of specimens. One minute after a given stimulation series, a further single stimulation was performed in order to check whether the cardiac response had or had not vanished, i.e. whether or not habituation had taken place. Habituation persistence was evaluated by measuring the time period a fly needed for showing a cardiac response to a successive stimulation.

The highest percentage of habituated flies (92 %) showed the lowest response threshold (1st group: 0.2 nM) (Fig. 1). A slightly lower number of specimens (83 %) exhibited a higher response threshold (2nd group: 2 nM), while the lowest percentage of habituated flies (64 %) showed the highest threshold value (3rd group: 20 nM). In the latter, habituation acquired after a series of six stimulations was retained for the shortest time period, with 36 % of them showing 3 h memory (Fig. 2). More than 50 % of specimens of the 2nd and 3rd groups showed 5 h and 7 h memory, respectively. On the other hand, flies trained to habituation after a series of three stimulations showed a maximum of 3 h memory, even in the case of specimens with the lowest response threshold (0.2 nM of the chemical).

The results show the existence of an inverse relationship between cardiac response threshold to olfactory information and response habituation, i.e. the greater the olfactory sensitivity, the more learning is induced. Moreover, greater sensitivity was found to be decisive in determining the longest period of memory retention and consolidation.

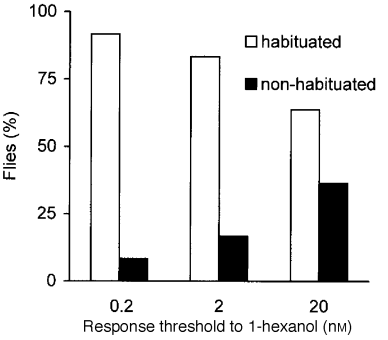

Figure 1. Habituation of the cardiac response to olfactory stimulation with 1-hexanol vapours in *Protophormia terraenovae* blowflies. Single flies of a group of 40 were trained to habituation with a series of six successive stimulations (one per minute) with the concentration of the chemical corresponding to the cardiac response threshold. Significant difference among all values $P < 0.05$, 2×2 χ^2 test.

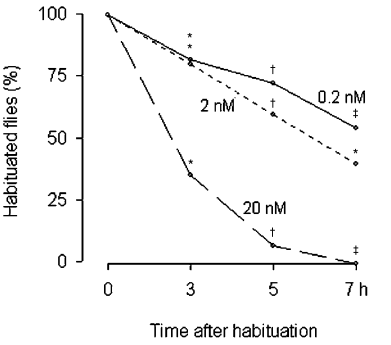

Figure 2. Habituation retention in blowfly groups in Fig.1. In each specimen, retention of habituation was evaluated by measuring the time period a habituated fly needed for showing a cardiac response to a successive threshold stimulation. Significant differences: *among different groups, $P < 0.05$, 2×2 χ^2 test; †among different groups, $P < 0.02$, 2×2 χ^2 test; ‡among different groups, $P < 0.02$, 2×2 χ^2 test.

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Localisation of C-nociceptor-activated spinal cord neurones that project to the ventrolateral periaqueductal grey in the rat

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Neurones in the ventrolateral periaqueductal grey (VL-PAG) are believed to co-ordinate changes in sensory, autonomic and

somatomotor parameters as part of passive coping strategies in response to inescapable pain (see Lumb, 2002). As part of on-going studies into the triggers of passive coping, the aim of the present study was to localise C-nociceptor-activated spinal cord neurones that project to the VL-PAG.

Experiments were a two-stage process. First, in five pentobarbitone-anaesthetised (Sagatal 60 mg kg^{-1}, I.P.) adult rats instrumented to record blood pressure, cholera toxin B (CTb; 150 nl) was injected at sites in the right VL-PAG at which previous injection of DL-homocysteic acid (50 nl, 0.05 M) evoked depressor responses. Animals were allowed to recover for 5 days to allow retrograde transport of CTb and then re-anaesthetised as before. In the second stage, 'slow' (2.5 °C s^{-1}) ramps (30–55 °C) of contact heat were applied (six times in each animal) to the dorsal surface of the left hindpaw to preferentially activate C-nociceptors (Yeomans & Proudfit, 1996). Two hours later, to allow time for expression of Fos protein, the animals were given an overdose of anaesthetic and perfused with 4 % paraformaldehyde. Sections (50 μm) of the PAG were processed immunocytochemically to visualise injection sites and 40 μm sections of the spinal cord were processed to visualise neurones labelled retrogradely and those in which Fos protein was evoked by the heat stimulus.

Fos-positive neurones were found throughout the dorsal horn and in the lateral spinal nucleus (LSN), with by far the largest numbers (724.2 ± 169.2 (mean ± S.E.M.); 76.6 % of total Fos-positive neurones) found in lamina I and 15.3 % in lamina II. Double-labelled neurones, i.e. those activated by C-nociceptors that projected to the VL-PAG were localised to two regions: lamina I in which 14.8 % of Fos-positive neurones were double labelled and LSN, in which, despite low numbers of Fos-positive neurones (16.2 ± 3.8), 41 % were double labelled. No double-labelled neurones were found in lamina II.

These data demonstrate that C-nociceptor input to the VL-PAG is relayed predominantly in lamina I and the LSN of the spinal dorsal horn. As such, these may represent critical pathways for triggering passive coping strategies in response to C-fibre-mediated pain.

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All procedures accord with current UK legislation.

Cooling-sensitive neurones from rat dorsal root ganglia express a distinct repertoire of voltage-gated currents

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It has recently been shown that cooling-sensitive (CS) neurones express a different pattern of voltage-gated ion channels than cooling-insensitive (CI) ones (Viana *et al.* 2002). Along with these authors and others (McKemy *et al.* 2002), we found using calcium imaging that about half of the cold receptors are also capsaicin sensitive (19/39, 49 %), which is very similar to the proportion of capsaicin-sensitive cells among cold-insensitive neurones (123/241, 51 %). As capsaicin sensitivity has been considered a hallmark of nociception, we tried to identify nociceptor-like properties among cold-sensitive neurones.

Adult rats were killed by CO_2 inhalation and decapitation, and dorsal root ganglion (DRG) neurones were dissociated and kept in culture for 1–3 days. Electrophysiological recordings were made from cells of diameter 20.0 ± 2.2 μm ($n = 92$) in the current-clamp and whole-cell voltage-clamp configurations. CS cells responded with bursts of action potentials on cooling to 18 °C. CI cells depolarised by less than 5 mV during the same cold stimulus. Data are presented as means ± S.E.M.

The following parameters were monitored: the magnitude of the hyperpolarisation-activated cation current (I_h), the inactivation time constant of the inward current and the presence of the fast-inactivating potassium current I_A. I_A was defined as the outward current elicited by a depolarising step, which inactivated with a time constant of less than 50 ms.

In agreement with Viana *et al* (2002), CS neurones expressed a higher level of I_h than CI ones (114 ± 24.2 pA, $n = 16$, compared with 70.2 ± 9.6 pA, $n = 39$, $P = 0.037$, Student's unpaired t test, significance level set at 0.05). In CS cells the inward current inactivated with a faster time course than in CI neurones (1.0 ± 0.1 ms, $n = 16$, compared with 2.2 ± 0.1 ms, $n = 39$, $P < 0.001$). Finally, I_A appears to be present in fewer CS neurones (6 of 16) than in CI ones (32 of 39).

CS neurones had action potentials of shorter duration (3.6 ± 0.3 ms, $n = 21$ compared to 4.5 ± 0.2 ms, $n = 38$, for CI neurones, $P = 0.02$) and fewer had an inflexion (8/21 compared to 27/38), whereas nociceptors have action potentials of long duration with an inflexion.

All CS cells were type 3, according to the classification proposed by Petruska *et al.* (2000). We could not identify nociceptor-like cells (type 1 and 2) among the CS neurones. As a conclusion, cold-receptors comprise a distinct subpopulation of rat DRG neurones.

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All procedures accord with current local guidelines.

Cerebellar projections to the posterior parietal cortex in the macaque monkey

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The posterior parietal cortex is thought to influence the motor cortex both directly, via cortical connections, and, indirectly, via subcortical pathways through the cerebellar loop.

In this study, carried out on seven macaque monkeys, we aimed to define the contribution of thalamic nuclei, target of cerebellar afferents, to projections towards the posterior parietal cortex. Experimental protocols complied with Italian law on the care and use of laboratory animals. Tracer injections were made under general anaesthesia (ketamine I.M. 15 mg kg^{-1}). Following survival time, animals were killed with an overdose of pentobarbital I.V. In two animals, injections of WGA-HRP in cerebellar nuclei labelled, in addition to motor thalamic nuclei,

nuclei Cl and Pcn and also some nuclei of the posterior thalamus (VLps, LP and Pul.m). In the posterior thalamus cerebellar afferents showed two patterns of termination: in VLps the labelling was strong and evenly distributed, whereas it showed a spot-like organization in LP and in rostro-dorsal part of Pul.m. In six monkeys tracers (WGA-HRP, fluorescent dyes) were injected into rostral and caudal areas of the superior parietal lobule (SPL; 3 injections) and in rostral, intermediate and caudal sectors of the inferior parietal lobule (IPL; 3 injections). Following all cortical injections the labelling in the thalamus formed a slab of marked neurons mostly involving posterior thalamic nuclei, but extending also in the caudal part of the motor thalamus. SPL areas receive a predominant projection from LP and additional projections from Pul.o, VLps, VLc, Pcn and Cl. However, rostral SPL injections labelled the ventral part of LP and VLc while caudal SPL injection produced a slab of labelling more dorsally located, involving dorsal LP and VLps. Following injections in IPL the most labelled nuclei were Pul.o and the ventral part of Pul.m. Additional projections originate from the intralaminar nuclei (Pcn and Cl) and from VLps, LP and VLc. Within these last nuclei, similarly to projections to caudal SPL, caudal IPL injection produced labelling more dorsally located than that following the rostral injection.

These findings suggest that the cerebellar input may reach both IPL and SPL through three different routes: motor thalamus, intralaminar nuclei, and posterior thalamus. However, none of these nuclei project exclusively to the parietal cortex, rather they project, although with different strength, to both frontal and parietal areas. Furthermore the observation that the cerebellar thalamic territory projecting to SPL appears to be larger than that projecting to IPL suggests a relevant link between SPL and cerebellum, and fits well with the recent proposal that, in respect to IPL, SPL is more involved in 'on-line' control of actions.

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All procedures accord with current National guidelines.