Raphe Magnus Neurons Help Protect Reactions to Visceral Pain From Interruption by Cutaneous Pain

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INTRODUCTION

Noxious stimulation at one site suppresses reactions to noxious stimulation at remote sites (Gear et al. 1999; Kalliomaki et al. 1992; Morgan et al. 1994). Such heterotopic antinociception requires a spino–bulbo–spinal loop, involving, at least in some cases, the raphe magnus (RM) and adjacent reticularis magnocellularis (NRMC) (Gear et al. 1999). RM and NRMC neurons project to the dorsal horn of the spinal cord and modulate both cutaneous and visceral nociceptive transmission (Fields et al. 1991; Lumb 1986; Mason 2001; Sandkuhler 1996). RM or NRMC activation typically suppresses, but can also facilitate, motor and cellular responses to noxious stimuli (Zhuo and Gebhart 2002; Zhuo et al. 2002). Inactivation experiments provide evidence for nociceptive-facilitating and -inhibiting cells (Kaplan and Fields 1991; Sandkuhler and Gebhart 1984), physiologically identified as ON and OFF cells, respectively (Fields et al. 1991; Porreca et al. 2002). ON and OFF cells are nonserotonergic, as are neutral cells that have an unclear role in nociceptive modulation (Gao and Mason 2000; Mason 1997; Potrebic et al. 1994).

Because noxious cutaneous stimulation inhibits nociception-inhibitory cells and excites nociception-facilitatory ON cells, an enhancement of subsequent nociceptive reactions is predicted after such stimulation. Yet, antinociception is more commonly observed. This paradox was previously noted and addressed experimentally, although no compelling cellular mechanism has emerged (Hernandez et al. 1994; Morgan and Fields 1994). Here, we use a specific form of heterotopic antinociception: the suppression of heat-evoked withdrawals by the noxious visceral stimulus colorectal distension (CRD). Advantages to this paradigm include the component stimuli (paw heat, CRD) both of which are repeatable, minimally invasive, and parametric. Additionally, this form of heterotopic antinociception reflects the fundamental dichotomy between behaviors evoked by noxious cutaneous (escapable) and visceral (nonescapable) stimuli, behaviors that are mutually exclusive (Lumb 2004). CRD-evoked withdrawal suppression is greatly reduced by cold block at C1 but unaffected by decerebration (Ness and Gebhart 1991b). To examine whether RM is required for this form of heterotopic antinociception, the suppression of heat-evoked withdrawals by CRD was examined before and after RM inactivation by microinjection of muscimol, a γ-aminobutyric acid type A (GABA_A) receptor agonist.

The efferent functions of ON and OFF cells derive from their responses to analgesic doses of opioids with ON cells inhibited and OFF cells excited (Barbaro et al. 1986; Fields et al. 1991; Mason 2001). Yet, individual ON and OFF cells are excited as well as inhibited by CRD (Brink and Mason 2003). Because Ellrich’s group demonstrated that cells that respond to different noxious stimuli in opposing directions often have atypical responses to opioids (Schnell et al. 2002), we tested the possibility that the response to opioids may be altered in ON and OFF cells with variant responses to CRD. Our results show that the response to noxious heat but not that to CRD predicts a cell’s response to opioids, further evidence that ON cells are nociception-facilitatory and OFF cells are nociception-inhibitory.

Finally, we hypothesized that the subset of OFF cells that are excited by CRD mediate CRD-evoked cutaneous antinocepc-

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tion and that the subset of ON cells excited by CRD (Brink and Mason 2003) may also contribute to CRD-evoked cutaneous antinoception by augmenting the cellular responses to CRD (Brink and Mason 2004; Ness and Gebhart 1988; Zhu and Gebhart 2002; Zhu et al. 2002). To test these hypotheses, we recorded the discharge of RM and NRMC cells during suppression of cutaneous heat-evoked withdrawals by CRD. If RM and NRMC cells actively suppress cutaneous nociception during CRD application, then their discharge during paired stimulus application should resemble their response to CRD alone. In contrast if these cells are not involved in suppressing cutaneous nociception but are simply receiving a reduced sensory input reflective of dorsal horn activity, then their discharge during paired stimulus application should be suppressed relative to their responses to CRD or heat alone.

METHODS

Surgery

Male Sprague–Dawley rats (n = 68, 250–500 g; Charles River, Portage, MI) were treated with atropine sulfate [40 μg in 0.1 ml, administered subcutaneously (sc)] and anesthetized with halothane. In early experiments, a Y-tube was inserted into the trachea, whereas in later experiments, halothane was administered through a nose cone adapted for the stereotaxic. Halothane was maintained at 1.8–2.0% in oxygen for the surgery. A catheter was inserted into the femoral artery to record blood pressure. Needle electrodes were placed into the thorax bilaterally to record the electrocardiogram, into the biceps femoris to record the electromyographic activity (EMG) of the hind limb muscles, and into the superficial abdominal musculature to record the hunching evoked by CRD. A craniotomy was made over the cerebellum and the exposed dura was cut. Core temperature was maintained at 36–38°C by a water-perfused heating pad. After the surgery, the halothane concentration was lowered to 0.8–1.0% and the animal was allowed to equilibrate for 1 h.

Electrophysiological methods

Tungsten metal electrodes (A-M Systems, Pullman, WA) were used for all experiments. Microelectrodes were lowered into the region of the RM and NRMC (P, 10.5–11.8 mm from bregma; L, 0.0–1.0 mm; V, 8.5–10.5 mm from cerebellar surface). Neurons were isolated by their spontaneous activity and each extracellular unit that was successfully isolated was studied. The unit waveform was acquired at 40 kHz by a CED Micro1401 interface (CED, Cambridge, UK). Spike2 acquisition software (CED) stored the time of the spike and 30 μs digitized points from the waveform. Individual waveforms were discriminated off-line using template-matching software.

Stimulation methods

Cells were tested for their responses to noxious paw heat and CRD. Heat stimuli were applied using a peltier device (Yale Instrumentation, New Haven, CT) placed on the footpad and toes of the hindpaw. The paw was affixed to the peltier platform (2 cm²) so that it was exposed to the full-duration stimulus. Each heat stimulus consisted of a 2- to 3-s ramp from 32 to 46–56°C with a 4-s plateau at the peak temperature. The peltier platform then ramped back down to 32°C over the course of 3–7 s. Between thermal stimuli, the peltier platform was maintained at 32°C.

To inflate the colon, the finger portion of a glove was secured onto the anus, to a point just past the external sphincter muscle. All distensions were applied for 20 s.

Experimental protocol

Because we previously demonstrated that serotonergic neurons respond weakly, if at all, to CRD, we did not study serotonergic cells (Brink and Mason 2003). Therefore cells that discharged very slowly and regularly—cells that are very likely to be serotonergic—were not recorded (Mason 1997). All other cells were tested with repeated (three to five) trials of noxious paw heat that were interleaved with CRD trials. All stimuli were separated by intervals of 5 min. After characterization of a neuron’s response to paw heat and CRD, trials of noxious paw heat alone were interleaved with paired CRD + heat trials. In paired CRD + heat trials, CRD was applied 10 s before the onset of the paw or tail heat stimulus. The paw heat stimulus was always presented coincident with the CRD stimulus because CRD-evoked suppression of withdrawals does not outlast the CRD stimulus (Ness and Gebhart 1991b).

In early experiments, all cells were tested with heat intensities to 56°C and CRD trials of 80 mmHg. In later experiments, the peak temperature of the cutaneous stimulus was chosen to be the minimum that reliably elicited a robust motor withdrawal and was typically 49 or 52°C and initial CRD stimuli were applied at an intensity of 60 mmHg. If a cell did not respond to the 60 mmHg CRD, the maximal intensity of 80 mmHg was applied. In a few cases, the intensity of CRD stimulation was lowered to 40 mmHg. The recording site for at least one cell per animal was labeled by injection of 20-μA hyperpolarizing current for 4 min.

DAMGO microinjections and cell recording

Rats were surgically prepared as described above. In addition, an anterior craniotomy was performed and a guide cannula (Plastics One, Roanoke, VA) was cemented into place over the right lateral ventricle. After equilibration at 1% halothane, a cell with an apparent response to noxious paw heat was isolated and characterized as described above; neutral cells were not studied in this experiment. DAMGO ([D-Ala²,NMePhe³,Gly⁵-ol]-enkephalin), 50 ng in 5 μl PBS, intracebroventricular [icv] administration) was then administered and CRD and paw heat trials continued at 5-min intervals. When at least three trials of CRD and paw heat were recorded after DAMGO administration, naloxone hydrochloride (0.4 mg in 1 ml; Abbot Laboratories) was injected intramuscularly (im) and followed by more trials of CRD and heat. In six rats, the effect of 5 μl PBS (vehicle) on baseline activity and a single trial of paw heat and CRD were tested before DAMGO administration. Recording and microinjection sites were recovered and plotted on standard sections.

Muscinol microinjections into RM

Animals were prepared with a guide cannula (Plastics One) into RM as well as with EMG electrodes as above. An injection cannula, filled with either saline or muscinol, was placed in the guide cannula. After a 1-h equilibration at 1–1.2% halothane, the motor reactions to at least four trials of paw heat alone alternating with at least three trials of paired CRD + heat stimulation were acquired. Muscinol (50 ng in 500 nl) or saline (500 nl) was injected in RM, over the course of 1 min. The injection cannula was left in place for the remainder of the experiment. Alternating heat alone and paired CRD + heat trials continued at 5-min intervals for 1 h after injection. Microinjection sites were recovered and plotted on standard sections.

The integrated rectified EMG activity for the 10 s after the heat stimulus was calculated for all trials. The percentage suppression for each paired trial was calculated as 1 – (CRD + heat/previous heat alone).
Histology

Animals were overdosed with 5% halothane and perfused with a fixative containing 4% paraformaldehyde and 7% sucrose in 0.1 M phosphate-buffered saline. The brain stem was removed, postfixed for 2–12 h, and then immersed in 30% sucrose in 0.1 M PBS. Coronal sections (50 μm) were cut on a freezing microtome. Sections were mounted on gelatin-coated slides and then stained with cresyl violet. Lesion sites were identified and recovered. Under ×50 magnification, the mediodorsal and dorsoventral distances from midline and the ventral midline edge of the section, respectively, were measured. Sites were assigned an anterior–posterior location by comparison of sections with a standard atlas (Paxinos and Watson 1986). Unlesioned sites were located by their stereotaxic distance from marked recording sites.

The nuclear boundaries used are modified from Newman (1985). We considered RM to include a region 300 μm wide centered on the midline and extending from the base of the brain to a point 1,500 μm dorsal, at levels from −11.6 to −10.4 mm relative to bregma. A small region between the pyramids (150 μm dorsal, 50 μm lateral on each side) with densely packed, small cells was considered to be raphe pallidus. NRMC was considered to include a region that stretched laterally from RM to the lateral edge of the pyramids and had a dorsal extent of 1,000 μm. Cells dorsal to the RM and NRMC were considered to be located in nucleus reticularis gigantocellularis.

Cellular analysis

Although cells with a slow and regular pattern of discharge that are highly likely to be serotonergic (Li and Bayliss 1998; Mason 1997; Wang et al. 2001) were not studied, an algorithm that physiologically identifies serotonergic and nonserotonergic cells was used to ensure that all studied cells were indeed nonserotonergic (Mason 1997). Previously described methods were used to classify cells as ON, OFF, or neutral (Leung and Mason 1998) and to characterize cell responses to CRD as excitatory, inhibitory, or unaffected (Brink and Mason 2003). Briefly, the mean and SD of the change in discharge rate across sequential 10-s bins was calculated for a period of no stimulation. Evoked changes were considered significant if they exceeded a threshold that was defined as 2SD. Using these criteria, cells were classified by their responses to noxious paw heat as ON, OFF, or neutral and separately characterized by their responses to CRD as excited, inhibited, or unaffected.

Analysis of physiological responses

Systolic blood pressure was calculated from the blood pressure recording and instantaneous heart rate was calculated from the inverse of the interval between QRS waves in the EKG. Customized software then converted blood pressure and heart rate from x, y pairs to arrays (10 values/s) using linear interpolation. Evoked EMG activations were quantified as the total rectified and integrated EMG recorded during the stimulus less the total EMG for a corresponding period before the stimulus.

Statistics

Each variable is expressed as a mean ± SE. Statistical tests were performed using Microsoft Excel (Redmond, WA) or SigmaStat (SPSS Science, Chicago, IL).

Results

Paired CRD + heat suppresses motor withdrawals from paw heat

Noxious CRD evoked activation of abdominal muscles (not shown; see Brink and Mason 2004) but not of hind limb musculature (Fig. 1A, left column). When applied alone, noxious paw heat evoked a robust motor withdrawal from the hind limb musculature (Fig. 1A, middle column). In paired CRD + heat trials (n = 289 trials), the hind limb withdrawal was suppressed by an average of 82% (paired t-test, P = 0.02; Fig. 1A, right column).

In several cases (n = 27), the effect of more than one pairing of CRD and heat intensity was tested. When a given intensity of CRD was paired with two or more intensities of heat that differed by 3°C (n = 18), the suppression was unchanged because the evoked reaction averaged 11 ± 4% (of the reaction to the previous heat) at the lower heat intensity and 16 ± 5% at the greater heat intensity. Similarly, increasing the CRD intensity by 20 mmHg while keeping the heat intensity constant did not change the amount of paw withdrawal suppression (n = 9). The paw withdrawal averaged 16 ± 5% when paired with a lower CRD intensity and 15 ± 8% when paired with a greater CRD intensity. Thus CRD suppression of the paw heat withdrawal was not sensitive to incremental changes in either CRD or heat intensity within the noxious range.

**Paired CRD + heat has little effect on cardiovascular reactions**

In contrast to the suppression of heat-evoked motor reactions during paired stimulation (Fig. 1B), the evoked heart rate increase during paired CRD + heat stimulation was greater, by
56%, than that evoked by heat alone (paired t-test, \( P < 0.001 \); Fig. 1C). The pressor reaction was significantly less, but by only 11%, during paired presentations of noxious CRD + heat relative to the reaction to heat alone (paired t-test, \( P = 0.03 \); Fig. 1D).

**CRD suppression of paw withdrawal is decreased after muscimol microinjection into RM**

To determine whether RM is required for CRD-evoked suppression of paw withdrawals, heterotopic antinociception was measured before and after either muscimol (50 ng in 500 nl) or saline microinjection (500 nl) into the medullary raphe. To isolate the effect of raphe on antinociception from raphe’s contributions to visceral and cutaneous nociception, data from animals where either the CRD or paw withdrawal magnitude changed by \( >20\% \) were omitted. For this reason, data from two animals where the reaction to CRD decreased after muscimol injection and one animal where the paw withdrawal decreased after saline injection were not included. Microinjections were focused in caudal RM at the caudal pole of the facial nucleus extending caudally to the rostral pole of the inferior olivary complex (from \(-2.2\) to \(-3.0\) mm caudal to interaural zero). Muscimol (\( n = 8 \)) microinjection decreased CRD-evoked suppression of the paw withdrawal from \( 63.9 \pm 3.6 \) to \( 49.5 \pm 8.5\% \) (\( P = 0.05 \), paired t-test). In contrast, suppression was not changed after saline microinjection (\( n = 4 \), \( P = 0.88 \), paired t-test).

**A cell’s response to noxious paw heat predicts its response to intracerebroventricular DAMGO**

To determine whether the RM cell response to opioids is indeed predicted by the response to noxious heat, even among cells with a different response to CRD, the responses of 38 neurons to supraspinally administered DAMGO were recorded. Cells were characterized by their responses to both noxious paw heat and CRD (Table 1) and then recorded after DAMGO (50 ng in 5 \( \mu \)l PBS, icv) and subsequent naloxone (0.4 mg, intraperitoneal) administration. Figure 2, A1–A3 shows that DAMGO simultaneously excited an OFF cell excited by CRD (Fig. 2A1) and inhibited two ON cells, one excited (Fig. 2A2) and one inhibited (Fig. 2A3) by CRD. All OFF cells were excited by DAMGO regardless of whether they were excited (\( n = 7/7 \)) or inhibited (\( n = 2/2 \)) by CRD (Fig. 2B). ON cells were significantly inhibited by DAMGO regardless of whether they were excited (\( n = 11/12 \)) or inhibited (\( n = 16/17 \)) by CRD (Fig. 2B). A two-way repeated-measures ANOVA confirmed the obvious impression from these data that the response to DAMGO was significantly different for cells with different responses to paw heat (\( P = 0.005 \)) but not for cells with different responses to CRD (\( P = 0.74 \)).

Naloxone decreased cell discharge, regardless of whether the OFF cell was excited (\( n = 6/7 \)) or inhibited (\( n = 2/2 \)) by CRD, and increased the discharge of most ON cells, again regardless of whether they were excited (\( n = 11/12 \)) or inhibited (\( n = 15/17 \)) by CRD (Fig. 2B). A two-way repeated-measures ANOVA showed that the response to naloxone was significantly different for cells with different responses to paw heat (\( P = 0.003 \)) but not for cells with different responses to CRD (\( P = 0.89 \)). Most cells were located within NRMC (\( n = 18 \)) or RM (\( n = 9 \)); the locations of 11 cells were not recovered.

**RM cells are heterogeneous with respect to resting discharge and responses evoked by noxious heat and CRD**

Cellular recordings were made from 85 nonserotonergic RM cells that were characterized as ON (\( n = 38 \); 45\%), OFF (\( n = 19 \); 22\%), or NEUTRAL (\( n = 26 \); 31\%). Two cells (2\%) could not be classified as the result of inconsistent responses to noxious heat or because the background firing rate was \(< 1\) Hz. The back-
As detailed here, we found that both on cells inhibited by CRD and off cells excited by CRD discharged similarly during CRD + heat as they did during CRD alone. Figure 3D shows average population responses of on cells inhibited by CRD during heat alone and during paired CRD + heat stimulation. There was a brief (3-s) period at the end of the heat stimulus when the CRD-evoked inhibition was partially relieved and the discharge returned to baseline levels (two-way repeated-measures ANOVA, \( P < 0.001 \), post hoc Newman–Keuls with a Bonferroni correction \( P < 0.05 \) marked by + signs). Off cells excited by CRD, like on cells inhibited by CRD, responded similarly to CRD alone and CRD + heat trials with only a brief and partial interruption (Fig. 3A; + signs as described above). Figure 4 shows one such example; the response of this off cell excited by CRD to paired stimulation was not distinguishable from its response to CRD alone and did not resemble the response to heat alone.

For cells that responded to heat and CRD in the same direction, it was difficult to distinguish whether the response to CRD + heat was the same or different from the response to CRD alone. Indeed there was no difference between the population responses to CRD presented alone and during pairing for either on cells excited by CRD (Fig. 3B) or off cells inhibited by CRD (Fig. 3C). However, as shown in the individual example shown in Fig. 5, this lack of quantitative difference in the number of spikes obscured, in at least some cases, a striking qualitative similarity between the responses to CRD + heat and to CRD alone. The response patterns to the two noxious stimuli when presented alone were quite different in form, making it obvious that the response observed during paired trials was of the same form as the response to CRD alone. This domination of the CRD response in paired trials was again coupled with a significant suppression of the motor withdrawal.

In a minority of pairings (\( n = 8 \)), CRD suppressed the paw heat-evoked withdrawal by \(<50\%\). In these cases, the cellular response during pairing differed significantly from the response to CRD alone. Figure 6 shows an example of incomplete suppression in which the cell’s responses to CRD + heat \((C)\) was different from the cell’s response to CRD alone \((A)\) but resembled the response to heat alone \((B)\). Because there were so few trials with \(<50\%\) withdrawal suppression, population averages as shown in Fig. 3 could not be calculated for all cell types.

![Image](https://example.com/image.png)

**FIG. 3.** Mean responses to CRD presented either alone (gray) or paired with heat (black) for pairings that produced \(>50\%\) suppression of the paw withdrawal. Responses were averaged for off cells excited by CRD \((A, n = 17)\), on cells excited by CRD \((B, n = 27)\), off cells inhibited by CRD \((C, n = 10)\), and on cells inhibited by CRD \((D, n = 26)\). Every other error bar is omitted and the error bars of the 2 traces are staggered for clarity. Mean discharge rate for the 10 s preceding stimulation is shown to the left of each ordinate axis; 20-s averages are illustrated as a line under the bottom axes. Because a range of final stimulus temperatures was used, the average noxious thermal stimulus was calculated and is shown below the CRD stimulus lines at the time of its average occurrence during paired trials. Every panel is 20 s in duration. Plus symbols in A and D represent points that differ significantly between the 2 trial types (2-way repeated-measures ANOVA, \( P < 0.001 \), post hoc Newman–Keuls with a Bonferroni correction, \( P < 0.05 \)).

Ground discharge rates of on, off, and neutral cells did not differ \((P = 0.44)\) and averaged 10.6 ± 1.3 spikes/s and ranged from 0 to 80.4 spikes/s. CRD excited \((n = 38; 45\%)\), inhibited \((n = 26; 31\%)\), or did not affect \((n = 20; 23\%)\) the discharge of recorded cells. The response of one cell \((1\%)\) to CRD could not be classified. As previously shown (Brink and Mason 2003), a cell’s response to noxious heat did not predict its response to CRD (Table 1).

**RM cells typically respond to paired CRD + heat as they do to CRD alone**

The discharge of 85 cells during 123 pairings of CRD and paw heat was recorded. In the case of 33 cells, the effect of multiple (two pairings, \( n = 30 \); three, \( n = 1 \); four, \( n = 2 \)) CRD + heat pairings were tested. Most pairings were effective in suppressing the motor withdrawal by \(\geq50\%\). To determine whether the suppression evoked by a pairing influenced the response of a RM cell to that pairing, two groups were analyzed. Pairings \((n = 106)\) that resulted in \(>50\%\) suppression of the motor withdrawal provided a median suppression of the motor withdrawal of 92%. In the case of a minority of pairings \((n = 8)\) the motor withdrawal was suppressed by only 20–50% (median suppression 42%). In an additional nine pairings, recording problems precluded analysis of the suppression.

To determine which cells may mediate CRD-evoked cutaneous antinociception, cellular discharge during paired stimulus application was compared with that in response to CRD alone for pairings that produced \(>50\%\) suppression. Determining whether the response of a cell to paired CRD + heat stimulation resembles the response to CRD alone is more straightforward for cells with opposing, and therefore clearly different, responses to CRD and heat presented alone. The numbers of cells recorded during heterotopic antinociception with each combination of responses to paw heat alone and to CRD alone are shown in Table 1.

![Image](https://example.com/image.png)

**FIG. 4.** Responses of an off cell that was excited by CRD to CRD alone \((80 \text{ mmHg})\; A\), heat alone \((56\; ^\circ\text{C})\; B\), and paired CRD + heat \((C)\). In each panel, the traces represent from top to bottom an example of cellular discharge from a single trial, mean cellular discharge across trials, mean rectified flexor EMG, and application of the heat and CRD stimuli. Discharge in the single trial is shown as instantaneous discharge and that in the average as mean firing rate within 250-ms bins. All traces are averages of 5 trials.
types. Still, three ON cells were recorded during incomplete suppression and a population average for these cells shows that their responses during paired stimulation were the same as those during heat stimulation alone (Fig. 7).

In several cases, the cellular responses during several different pairings were recorded. Figure 8 shows one such recording in which the suppression of heat-evoked withdrawals decreased from 90 to 80% while recording a cell’s responses to CRD (40 mmHg) + increasing intensities of heat (A, 49°C; B, 52°C; C, 55°C). In parallel with the decrease in motor suppression, a heatlike response emerged as indicated by the arrows in Fig. 8, E–G. The heatlike response present at 80% suppression was more evident than it was at 90% but still a mere fraction of the response to heat stimulation alone. This result suggests that the similarity between the response to CRD + heat and heat alone grades with the degree of motor suppression produced by any given pairing.

Recorded cells were located in RM and NRMC

For this cohort of cells, recording sites were previously illustrated (Fig. 1 in Brink and Mason 2003; Fig. 9 in Brink and Mason 2004). Cells were concentrated within RM (n = 59) and NRMC (n = 21) from the rostral pole of the inferior olive to the level of the facial genu, with the largest number being concentrated at the level of the facial nucleus.

DISCUSSION

CRD suppresses motor withdrawals but not cardiovascular reactions to paw heat

Unlike the motor withdrawal, the blood pressure and tachycardic reactions evoked by noxious paw heat were minimally altered by paired CRD + heat presentation. Paired CRD + heat continued to evoke rather than suppress cardiovascular reactions. This was true regardless of whether motor withdrawal suppression occurred and regardless of how the RM cell responded, implying that CRD input critical to evoking cardiovascular reactions splits from the pathway that gives rise to somatomotor reactions at an early point (Fig. 9). The occurrence of withdrawal suppression with maintained, and sometimes enhanced, reactions rather than depressed somato-sym pathetic reactions may be adaptive during freezing, a common response to threat in the rat (Blanchard et al. 2001). For example, when a rat encounters a predator at close proximity, the rat becomes immobile but is in a state of heightened skeletal muscle tone, sympathetic arousal, and vigilance.

RM contributes to heterotopic antinociception

Microinjection of muscimol but not saline significantly reduced the CRD-evoked suppression of noxious heat-evoked paw withdrawals by about 15%. This is highly consistent with the 15% difference between mean tail-flick latencies during on cell bursts versus during off cell bursts (Heinricher et al. 1989). Thus at least in the conditions studied—the lightly anesthetized rat with acute surgery—RM ON and OFF cells alone have only a modest influence on withdrawal movements. These modest RM contributions likely sum with antinociceptive influences arising from additional brain stem and spinal regions such as the caudal medullary subnucleus reticularis dorsalis (Gall et al. 1998), the first two cervical segments (Chandler et al. 2002), and lumbosacral segmental neurons, to completely suppress cutaneous stimulation-evoked withdrawals during CRD. Although tradition would suggest that RM modulates the reactions to paw heat primarily at the level of the dorsal horn, our experiments do not rule out an additional influence of RM on brain stem circuitry. Further, our experiments do not speak to the location or nature of the cellular target of RM modulation.

Raphe’s influence on heterotopic antinociception may be greater than implied by our inactivation experiments for several reasons. First, only a single microinjection of 500 nl was delivered, insufficient to affect the entire extent of the nociceptive modulatory region of the medullary and caudal pontine raphe (Sandkuhler and Gebhart 1984). Second, the muscimol injections used in the present study indiscriminately inhibited all RM and NRMC cell types, including ON cells that likely facilitate the reaction to CRD as well as OFF cells important to the suppression of paw withdrawals from noxious heat. Because both pro- and antinociceptive influences were presumably silenced, a small net effect on CRD-evoked antinociception is not surprising. We tried to control for injections that suppressed ON cell facilitation by omitting experiments with >20% reduction in the reaction to CRD after muscimol. However, given the nonlinearities inherent to spiking cells, it is possible that even a small change in the behavioral reaction to CRD is accompanied by a large reduction in downstream effects such as CRD-evoked OFF cell excitation. Finally, a model by which individual sites in the brain provide discrete functions that are either added or subtracted from the total is likely less accurate than a paradigm in which a number of sites,
which individually have only a small effect, interact synergistically to exert a large and complete effect. RM and NRMC cells have the appropriate connections and functional capacity to contribute to nociceptive modulatory tone in an ongoing fashion, a contribution that is not obviated by partially redundant contributions from additional forebrain, brain stem, and spinal sites.

*The response of a medullary raphe cell to noxious cutaneous stimulation accurately predicts the cell’s response to an opioid and therefore its functional role*

We show here that the response of a RM neuron to noxious cutaneous but not noxious visceral stimulation predicts that cell’s response to a centrally administered mu-opioid receptor agonist. The possibility that ON and OFF cell populations have heterogeneous responses to opioids has been advanced by Ellrich’s group, who reported that the discharge of five of six OFF cells with at least one excitatory or nonresponse to noxious stimulation was suppressed by morphine and one of six ON cells with at least one inhibitory or nonresponse to noxious stimulation was excited by morphine (Schnell et al. 2002). They also found that five of nine neutral cells that responded to at least one noxious stimulus also responded to morphine. In contrast, we have found that the responses of ON and OFF cells to opioid administration are predicted by the response to noxious paw heat but not by the independent response to CRD. Different rat strains and anesthetics were used and may account for the discrepancy between the two studies. Alternatively, it is possible that a small minority of RM cells have atypical responses to opioids and that cells with atypical responses to opioids are more likely to have heterogeneous responses to noxious stimulation than cells with the typical opioid response profile.

Because the descending antinociceptive influence from RM depends on activation, RM cells that exert antinociceptive effects on spinal neurons must be activated by opioids (Fields et al. 1991). Thus a cell’s response to opioids is critical to predicting that cell’s function. Noxious visceral stimulation excites a mixed population of nociception-facilitating ON cells and nociception-inhibiting OFF cells. Thus RM neurons may either facilitate or inhibit nociceptive transmission in response to noxious visceral stimulation. This conclusion rests on two assumptions: 1) the activation of some but not all members of an RM cell population can exert a behavioral effect, and 2) the activation of cells with an opposing function is not sufficient to negate a behavioral effect. Evidence for both of these assumptions exists. First, microstimulation in RM at very low stimulation intensities modifies nociceptive responsiveness (Hentall et al. 1991; Zhuo and Gebhart 1997). This was previously used to estimate that the minimum number of OFF cells needed to suppress nociception is <100 (Hentall et al. 1984). Because the stimulation threshold for facilitation is about an order of magnitude less than that for nociceptive inhibition (Zhuo and Gebhart 1997), it appears that activation of <10 ON cells is sufficient to facilitate nociception. Thus excitation of a minority of either the ON or OFF cell populations will produce behavioral changes.

On cell activation is clearly not sufficient to cancel the effects of OFF cell activation. This obtains from the consistent observation that RM stimulation typically suppresses nociception but at intensities well above those needed to evoke facilitation. Although OFF cell activation can suppress nociception even when ON cells are concurrently active, the only evidence to support the reverse stems from studies of animals in persistent pain. During such situations, both ON and OFF cells are discharging (Montagne-Clavel and Oliveras 1994) and both nociceptive facilitation and inhibition are evident (Coutinho et al. 1998). The applicability of the persistent pain condition to the normal condition is questionable, leaving open the question of whether ON cell activation can facilitate nociception when OFF cells are also discharging under normal circumstances.

**CRD ON and off cells contribute to heterotopic antinociception**

When CRD successfully suppressed noxious heat-evoked paw withdrawals, the discharge of RM cells resembled the response to CRD alone. This was clearly true for OFF cells excited by CRD and ON cells inhibited by CRD, populations whose responses to CRD and heat can be easily disambiguated. It also appeared to be true for at least some OFF cells inhibited by CRD and ON cells excited by CRD. However, in these latter populations, responses to CRD and heat were in the same direction and only sometimes could be distinguished based on pattern rather than on total number of spikes. Although it is possible that distinct patterns, such as those shown in Fig. 5, do not provide distinct messages to postsynaptic cells, it is also possible that differently patterned spike trains release different...
Cardiovascular reactions evoked by paw heat are preserved during pairings of paw heat with CRD. At the very least, this demonstrates that more than one "channel" for noxious information exists so that even when the motor reaction to paw heat is suppressed, nociceptive input from the paw reaches central circuits. This implies that paw-related information is available within the CNS and possibly within RM and NRMC, regardless of whether the paw stimulus elicits a motor reaction. Our finding that inactivation of RM decreases the CRD-evoked suppression of paw withdrawals further supports the idea that paw-related information reaches RM and that RM cell discharge reflects efferent function. In other words, RM does not respond to paired CRD + heat stimulation as it does to CRD alone simply because of reduced sensory input reaching RM.

To participate in CRD-evoked antinociception, an RM or NRMC cell must be excited by CRD. Once excited, activity in this cell must affect cutaneous nociceptive transmission, a criterion that is clearly met only by ON and OFF cells. Largely because of their excitatory response to analgesic doses of opioids, OFF cells are thought to suppress nociceptive transmission (reviewed in Mason 2001). In contrast, ON cells appear to play a pronociceptive role (Porreca et al. 2002). Thus RM and NRMC cells proposed to participate in CRD-evoked suppression of cutaneous withdrawals are limited to ON and OFF cells that are excited by CRD. Although we do not propose that cells inhibited by CRD or NEUTRAL cells responsive to CRD participate in the proposed model, our data do not preclude either possibility.

Our model is distinct from the DNIC model

LeBars, Villanueva, and colleagues studied reduction in the response of wide dynamic range spinal neurons to C-fiber stimulation evoked by a heterotopic noxious stimulus such as tail or muzzle immersion in hot water. They termed such noxious stimulus-evoked reductions in nociceptive responses as "diffuse noxious inhibitory controls" or DNIC. DNIC evoked by a noxious cutaneous stimulus depends on a spino–bulbo–spinal loop but is not blocked after large lesions of RM and NRMC (Bouhassira et al. 1993). These lesion studies have not been repeated using a form of DNIC evoked by visceral stimulation, precluding any direct comparison with the present study.

Our proposed model cannot explain heterotopic antinociception elicited by noxious cutaneous stimulation

Even though our model provides a mechanism for visceral stimulation-evoked suppression of cutaneous nociception, it fails to explain the suppression of cutaneous nociception by cutaneous noxious stimulation. For example, our model does not explain the suppression of CRD reactions by noxious cutaneous heat (Ness and Gebhart 1991a), which occurs albeit less robustly than the CRD-evoked suppression of cutaneous nociception. It is possible that RM plays no role in cutaneous–cutaneous heterotopic antinociception, which may be supported instead by a DNIC- or contrastlike mechanism dependent on more caudal regions of the medulla (Gall et al. 1998). DNIC has been interpreted as a reduction of spatial contrast between segmental and extrasegmental nociceptive inputs. The spinal neuronal response to noxious stimulation is also de-
increased when the stimulation area is increased (Bouhassira et al. 1995). Given the dependency of cutaneous nociception on spatial summation and contrast versus the poor localization of visceral nociception, it is unlikely that heterotopic antinociception evoked by visceral stimulation depends on a DNIC-type mechanism. Moreover, although the inhibition of nociceptive neuronal responses and behavioral reactions by CRD has been termed “DNIC” by some, there is no evidence regarding the bulbular site important for this particular form of heterotopic antinociception.

The suppression of reactions to cutaneous stimuli by noxious visceral stimulation guards the recuperative reactions to visceral stimulation from interruption

We show here that a subset of RM off cells is a strong candidate to contribute to the CRD-evoked suppression of noxious heat-evoked withdrawals. The population of RM off cells that is excited by CRD is uniformly excited by supraspin al administration of opioid receptor agonists, further supporting an antinociceptive function. In our model, excitation of this cell population by CRD results in the suppression of noxious cutaneous input from the paw. RM and NRMC cells project to all levels of the spinal cord and activation in this region suppresses withdrawals evoked by noxious cutaneous stimulation applied anywhere on the body or head surfaces. Therefore the subpopulation of off cells that is excited by CRD would be expected to limit motor reactions to all noxious cutaneous stimuli during conditions of visceral pain. Such a mechanism may serve to maintain an organism’s focus on guarding and recuperative reactions to visceral pain and decrease distractions evoked by slight cutaneous injuries that are unlikely to be life-threatening. Consistent with this idea, patients that suffer from persistent visceral pain, irritable bowel syndrome, or Crohn’s disease have elevated thresholds to noxious cutaneous stimulation (Cook et al. 1987).

Suppression of cutaneous nociception by noxious visceral stimulation allows for the reactions to the visceral stimuli to dominate. Noxious cutaneous stimuli elicit sympathetic and somatomotor activation to support active fighting or escape. In contrast, their visceral counterparts evoke quiescence and parasympathetic activation (Lumb 2004). These starkly different reactions motivated Lewis to comment that “the difference in qualities of skin pain and of deep pain is so clear and each belongs exclusively to the corresponding structures that it would perhaps seem unsafe to class both together under the one unqualified term pain” (Lewis 1942). By suppressing noxious cutaneous inputs, antinociceptive RM cells that are excited by opioids and by noxious visceral stimulation ensure that the quiet recuperation elicited by visceral stimulation can continue uninterrupted, even in the presence of cutaneous inputs that would normally elicit activity.

GRANTS

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