Physiological Survey of Medullary Raphe and Magnocellular Reticular Neurons in the Anesthetized Rat

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Leung, Cynthia G. and Peggy Mason. Physiological survey of cell classes based on their activity before withdrawal from noxious thermal stimulus in the lightly anesthetized rat. J. Neurophysiol. 80: 1630 ± 1646, 1998. According to this original description, OFF cells are excited, ON cells inhibited, and NEU-neurons were recorded in rats lightly anesthetized with isoflurane. All cells that were isolated successfully were studied. After recording background discharge, the neuronal response to repeated noxious thermal and noxious mechanical stimulation of the tail was recorded. Most cells were identified as nonserotonergic by their irregular or rapid background discharge pattern. Because the spontaneous discharge of most RM nonserotonergic cells contained pauses and bursts, a comparison between the change in rate evoked by tail heat and the range of rate changes that occur spontaneously was used to classify cells. The mean responses of ON and OFF cells were more than four times the standard deviation of the changes in rate observed spontaneously. ON cells were excited in 86% of the tail heat trials tested. Similarly, OFF cells were inhibited in 97% of the noxious tail heat trials tested. The heat-evoked changes in ON and OFF cell discharge varied over more than two orders of magnitude and were greater in cells with greater rates of background discharge. The heat-evoked responses of ON and OFF cells had durations of tens of seconds to minutes and were always sustained beyond the visible motor response. Most ON and OFF cells responded to noxious tail clamp in a manner that was similar to their response to noxious heat. Most of the NEUTRAL cells that were responsive to noxious heat were responsive to noxious tail clamp. A minority of ON, OFF, and NEUTRAL cells responded to innocuous brush stimulation with weak, transient responses. Although many cells discharged too infrequently to be classified, units with physiological properties that were different from those described above were rare. In conclusion, most RM and NRMC cells belong to three nonserotonergic physiological cell classes that can be distinguished from each other by the consistency, not the magnitude, of their responses to repeated noxious thermal stimulation. Because most of the heat-evoked change in ON and OFF cell discharge occurs after the conclusion of the initial motor withdrawal, ON and OFF cells are likely to principally modulate the response to subsequent noxious insults.

INTRODUCTION

Neurons in the pontomedullary raphe magnus (RM) and adjacent nucleus reticularis magnocellularis (NRMC) project to the dorsal horn and are thought to participate in the modulation of spinal nociceptive transmission (Fields et al. 1991). Fields and colleagues introduced a system that divides neurons in the RM and NRMC into three physiological cell classes based on their activity before withdrawal from a noxious thermal stimulus in the lightly anesthetized rat (Fields et al. 1983). According to this original description, OFF cells are inhibited and ON cells excited before the withdrawal reflex elicited by noxious stimulation. NEUTRAL cells were thought to be unaffected by such stimuli. Cells in these physiological classes also exhibit differential responses to opioids. OFF cells are excited, ON cells inhibited, and NEUTRAL cells unaffected by analgesic doses of opioids administered either systemically or by supraspinal microinjection (Barbaro et al. 1986; Cheng et al. 1986; Heinricher et al. 1992). A number of such studies have led to the idea that ON and OFF cells, which project to the superficial dorsal horn, facilitate and inhibit, respectively, spinal nociceptive transmission (Fields et al. 1991, 1995).

Although many investigators use the Fields classification system (Fields et al. 1991; Hentall et al. 1993; McGraugthy and Reinis 1993; Morgan and Heinricher 1992; Rosenfeld et al. 1990; Thurston and Randich 1992), there is no formal consensus on the criteria used. The few attempts to quantify the magnitude and variability of the noxious heat-evoked response across multiple tail heat trials have yielded data that differ from the original profile of ON, OFF, and NEUTRAL cell physiology. For example, one group of investigators reported that RM and NRMC neurons sometimes respond after the noxious heat-evoked reflex and that many RM neurons consistently respond to noxious heat even when no reflex is elicited (Thurston and Randich 1992). Other investigators reported that the magnitude or presence of the neuronal response to various stimuli may depend on the level of background activity at the onset of the trial (Blair and Evans 1991; Morgan and Heinricher 1992).

Because neurons that do not clearly fit into the ON or OFF cell classes often are excluded from study, the frequency and pattern of physiological properties among RM and NRMC cells is unknown. Therefore it is not possible to evaluate how completely the Fields model represents the neuronal cell types present in RM and NRMC. The present study was undertaken to provide a quantitative description of the physiological characteristics of each cell type as well as an estimate of the relative proportions of each physiological cell type in the anesthetized rat.

METHODS

Surgical preparation for acute recordings

Male Sprague-Dawley rats (240–400 g; Sasco, Madison, WI) were anesthetized initially with halothane. A tracheal catheter was
inserted and connected to an open breathing circuit for administration of isoflurane. Inspired and endtidal isoflurane concentrations were monitored using a multichannel infrared analyzer (Datex Capnomac, Madison, WI). Throughout surgery, deep anesthesia was maintained with 1.8–2.0% isoflurane in oxygen. Core body temperature was maintained at 37–38°C by use of a water-perfused heating pad and a plastic cover over the rat. A catheter was inserted into the femoral artery for recording blood pressure, and electromyographic (EMG) electrodes were placed transcutaneously in the paraspinal muscles. A small midline craniotomy was made just posterior to the lambdoid suture for the introduction of recording microelectrodes.

**Surgical preparation for chronic recordings**

A second group of rats (400–500 g) was prepared with miniature microdrives for chronic recording (unpublished data). Briefly, rats were anesthetized with pentobarbital sodium (Nembutal, 55 mg/kg) and placed in a stereotaxic apparatus. Bone screws were inserted through the skull for electroencephalographic recording, and stranded stainless steel wires were inserted through the biceps femoris and the deep muscles of the neck for EMG recording. A microdrive base and guide tube were affixed to the skull overlying the RM for extracellular unit recording. All electrodes were attached to a miniature connector, which was affixed to the skull with dental acrylic. Chronically prepared rats were used in the experimental protocol after ≥1 wk of recovery.

**Experimental protocol**

Both acutely and chronically prepared rats were studied only in the anesthetized condition. Rats were anesthetized initially with halothane and then maintained on isoflurane administered via a nose cone. Chronically prepared rats were not restrained or held in a stereotaxic holder, whereas acutely prepared rats were placed in a standard stereotaxic apparatus. Rats were placed on top of a water-perfused heating pad, and core body temperature was maintained at 37–38°C. EMG electrodes were placed transcutaneously in the paraspinal muscles.

After allowing rats to equilibrate to 1.10–1.25% isoflurane for ≥30 min, a metal microelectrode was introduced into the region of the RM and NRMC (P 0.8–2.6 mm from interaural zero, L 0.0–1.0 mm, V 8.5–10.5 mm from the cerebellar surface). In the case of chronically prepared rats, electrodes were introduced via a microdrive-microelectrode assembly (Biela Idea Development, Anaheim, CA) attached to the microdrive base. No search stimulus was used, and only units with background discharge were studied. To achieve as complete a sample as possible, every extracellular unit that was isolated successfully was studied. Each extracellular unit was discriminated off-line (Spike2, CED).

**Criteria for acceptable experiments**

One experiment (4 cells) was rejected because the rat exhibited extreme variation in blood pressure (standard deviation >10 mmHg) within a period of steady-state anesthesia. Several other experiments were terminated when the rat displayed obvious respiratory difficulty. Cells (n = 6) were excluded from analysis if the recovered recording site was outside of the boundaries determined to encompass the RM and NRMC region. Because <10% of recording sites were found to be outside the RM and NRMC region, 15 cells for which recording sites were not found were included in the analysis. For each of these 15 cells, the recording depth was in the range (9.0–10.5 mm) that was reliably associated with RM or NRMC locations.

**Analysis and cell classification**

Most cells were assigned to one of four physiological cell classes according to their background and noxious-evoked discharge properties. First, cells were classified as pSHT (physiologically identified as serotonergic) or non-pSHT (physiologically identified as nonserotonergic) using a previously described algorithm based on the rate and variability of the background discharge (Fig. 1) (Mason 1997). The probability of misclassification using this method is <10%. In the present study, the background discharge of isolated cells was recorded for 15 min and the mean (x) and standard deviation (SDx) of the interspike intervals (ISI) were calculated from this recording. For each cell, the value of the function, y(x, SDx) = 146 – x + 0.98 ∗ SDx, was calculated. All cells with a negative function value were classified as pSHT and those with a positive function value were classified as non-pSHT.

Discharge regularity was quantified by calculating the coefficient of variation of the interspike interval (CVISI). Extremely regular discharge will have a CVISI close to zero, whereas bursting discharge will have a CVISI greater than unity (Wilbur and Rinzel 1983).

To determine whether noxious heat-evoked changes in discharge were different from those occurring spontaneously, the variability
FIG. 1. Rate (abscissa) and regularity (ordinate) of the background discharge recorded from medullary raphe magnus (RM) and adjacent nucleus reticularis magnocellularis (NRMC) units. Two unclassified cells with mean interspike intervals (ISIs) of $>10$ s are not illustrated. A line representing the discriminant function $y(x, s) = 0$ defines the optimal linear boundary between physiologically identified as serotonergic (p5HT) and physiologically identified as nonserotonergic (non-p5HT) cells and is illustrated on this graph.

in background discharge was measured. The background discharge record was divided into 10-s bins, and the change in discharge rate between sequential 10-s bins was calculated. The variability of the background discharge then was quantified as the standard deviation of the change across all bins in the background discharge record and normalized to impulses/second ($SD_{10s}$). The magnitude of unit responses to peltier heat stimulation was calculated as the difference in unit firing rates (in imp/s) before and after the stimulus was applied ($10$ s before and $2-12$ s after stimulus onset). Heat-evoked increases in discharge that were $>2$ $SD_{10s}$ were considered excitatory responses and evoked decreases that were $>2$ $SD_{10s}$ were considered inhibitory responses. This method for detecting responses allows us to take into account the spontaneous ‘‘burstiness’’ of RM cells and to have confidence, at a $P < 0.05$ level, that an evoked change is unlikely to have occurred spontaneously.

A decrease in discharge was considered an inhibitory response only if it was a decrease of $\geq 2$ $SD_{10s}$. This criterion then presented a problem in cases when a cell was not discharging at a rate $\geq 2$ $SD_{10s}$ before the tail heat stimulus. Thus for such cells, the change in the maximum ISI was calculated. This change in maximum ISI was considered to be an inhibitory response if it exceeded 2 $SD_{10s}$ (see preceding text). As with the previous method, this method allowed us to take into account the natural variability of RM cell discharge and to have confidence, at a $P < 0.05$ level, that an evoked response was unlikely to have occurred spontaneously.

To classify non-p5HT cells, the percentage of excitatory and inhibitory responses to tail heat stimulation were calculated for each cell. Because ON and OFF cells are only thought to respond to stimulation that elicits a withdrawal (Fields et al. 1983), data from trials in which the cell did not respond and no withdrawal occurred were not included in these calculations. Cells that were excited by $\geq 50\%$ of heat applications were considered ON cells. Cells that were inhibited by $\geq 50\%$ of heat applications were considered OFF cells. Cells with $<50\%$ excitatory or inhibitory responses were classified as NEUTRAL cells. A minimum of three trials was required for classification. Therefore, cells that were not ON cells and did not discharge at rates of $\geq 1$ Hz before at least three tail heat trials (that evoked withdrawals) were not classified.

Three cells that would fit into the unclassified ($n = 1$) or NEUTRAL ($n = 2$) categories were noted to have consistent response patterns that deviated from those of ON, OFF, or NEUTRAL cells. These three cells then were categorized as ‘‘other’’ cell types and are described later.

The onset of the OFF cell pause was defined as the time of the action potential just before the response period’s maximal ISI. For cells that were inactive before the tail heat stimulus, the onset of the ON cell burst was defined as the time of the first action potential in the evoked burst. For cells that were discharging before the tail heat stimulus, the onset of the ON cell burst was defined as the inflection point in a plot of instantaneous frequency (Churchward et al. 1997).
Both systolic pressure and heart rate were calculated from the blood pressure recording in acutely prepared animals. The peak pressure during each heart beat defined the systolic pressure. Because pulse pressure did not change during the experiments, systolic pressure was adequate to describe changes in blood pressure. Heart rate was calculated from the inverse of the interval between heart beats and expressed as beats per minute (bpm). For each tail heat application, the changes in heart rate and blood pressure were calculated as the maximum (or minimum) value during the period 2–12 s after the tail heat onset less the mean value during the 10 s preceding the tail heat application.

Statistics
Each variable is expressed as a mean ± SE. Statistical tests were performed using Microsoft Excel 5.0 (Redmond, WA).

Histology
The recording site of the last cell recorded from each animal was lesioned by applying 20 µA of anodal current for 3–4 min. All other recording sites were calculated based on their stereotaxic distance from the marked recording site. Animals were perfused with a fixative containing 4% paraformaldehyde and 7% sucrose in 0.1 M phosphate buffer at pH 7.4. Transverse sections (80 µm) were cut on a freezing microtome, mounted on gelatin-coated slides, and stained with cresyl violet. Recording sites were examined microscopically and plotted onto standard sections.

Results
Proportions of cell types
A total of 136 RM and NRMC neurons were recorded in the anesthetized rat. Because a smaller number of p5HT cells (n = 8; 6%) were recorded than would be expected by their observed proportion in anatomic studies (15–20%) (Potrebic et al. 1994), it is likely that serotonergic cells were sampled inadequately. Therefore the percentages of ON, OFF, NEUTRAL, and unclassified cells are reported as a proportion of the total number of non-p5HT cells (n = 128). Overall, cells were classified as ON (n = 49, 38%), OFF (n = 30, 23%), or NEUTRAL cells (n = 21, 16%). A proportion of the cells recorded could not be classified (n = 25, 20%) and three cells (2%) had physiological characteristics different from any of the established cell types.

Background discharge patterns
Using a function that discriminates p5HT from non-p5HT cells (Mason 1997), eight p5HT cells were identified in the present sample (Fig. 1). The mean rate of discharge of the p5HT cells was 2.0 ± 0.5 Hz. The CV of p5HT cells ranged from 0.29 to 0.55, reflecting the observation that p5HT cell discharge does not contain bursts of activity and is also not “clock-like” in its regularity (Mason 1997).

The background discharge rate of ON (n = 49), OFF (n = 30), and NEUTRAL (n = 21) cells averaged 10.3, 15.8, and 15.6 Hz, respectively. As previously described (Barbaro et al. 1989; Leung and Mason 1995), the discharge of most of these cells was highly irregular, consisting of alternating bursts of activity and periods of inactivity (see Figs. 4A and 5A). As expected from the irregularity in background discharge, the CV of most ON (39/49), OFF (24/30), and NEUTRAL (13/21) cells was greater than one (Wilbur and Rinzel 1983) (Fig. 1).

A subset of ON (n = 7), OFF (n = 4), and NEUTRAL (n = 5) cells discharged regularly (CV < 0.50). The background discharge of these cells averaged 30.0 ± 6.3 Hz and contained no bursting activity, which was reflected in their mean CV of 0.30 ± 0.02. As detailed later, three of four regularly discharging OFF cells also differed from all other members of their class by responding to noxious heat and pinch with small decreases in discharge rate instead of frank pauses of >1 s. The CV of eight cells (3 ON, 2 OFF, 3 NEUTRAL) was between 0.5 and 1.0, reflecting discharge that was irregular but lacked pauses of >5–10 s. The background discharge of these eight cells averaged 7.5 ± 1.2 Hz.

Twenty-two cells could not be classified because of their slow (mean rate: 2.2 ± 0.5 Hz) and irregular discharge. Almost all such cells (21/22) had a CV of ≥ 2, and 17 of these cells had a CV > 5. The one cell in this group with a CV < 1 discharged only three action potentials during the entire 15-min period of background discharge recording.

Responses to noxious tail heat
ON CELLS. The response thresholds (± 2 SD) of ON cells (n = 49) varied from 0.5 to 49.5 Hz and averaged 6.8 ± 1.3 Hz (Fig. 2). The mean ON cell discharge increase evoked by tail heat was 20.5 ± 4.0 Hz and ranged over two orders of magnitude from 0.8–132.8 Hz. Thus in the 10 s after the onset of tail heat stimulation, ON cells had a mean of 205 more action potentials than during the 10 s preceding the tail heat stimulus. ON cells with greater background discharge rates had larger heat-evoked responses (Fig. 3). ON cells were excited by 86 ± 2% of the noxious tail heat trials (Fig. 2). Most ON cells (27/49) were excited by every tail heat stimulation, and 13/49 ON cells were excited by all but one trial. Only 2 of the 49 ON cells were inhibited by a single trial of tail heat each (Fig. 2).

The heat-evoked changes in discharge in ON cells were compared with those observed in NEUTRAL cells with an average increase in discharge. Although the mean change evoked in ON cells was significantly different from that evoked in this subset of NEUTRAL cells (t-test, P = 0.04), there was overlap between the values observed for the two groups (Fig. 2). Thus the tail heat-evoked responses of ON cells could not be distinguished from those of all other RM and NRMC cells by their magnitude and direction alone.

In the case of most ON cells, the peak discharge rate evoked by tail heat was similar to that recorded during background conditions (Fig. 4, A and B). Therefore, the change in rate evoked by tail heat was largest when the trial was initiated during a period of low discharge and smallest when the trial was initiated during a period of high discharge (Fig. 4B). For the ON cell population, the tail heat-evoked discharge was significantly greater when the cell discharged at 0–10% of maximal rates than when it discharged within 10% of maximal rates (t-test, P < 0.005). This reduction in the evoked change when an ON cell was bursting at the time of the heat onset sometimes resulted in trials where no excitatory response occurred, despite an evoked motor response (Fig. 4B). The latency to the change in discharge evoked by noxious...
heat was determined for all ON cells. For trials where an ON cell was inactive for ≥2 s before the evoked burst (n = 93 trials), the mean latency was 3.06 ± 0.10 s after tail heat onset. For trials where an ON cell was active before the evoked burst (n = 116 trials), the latency to burst, estimated from the inflection point in the interspike intervals, averaged 2.94 ± 0.08 s after tail heat onset. The latencies to respond during trials when the cell was active or inactive before the trial onset were not different (t-test, P > 0.3).

OFF CELLS. For OFF cells (n = 30), the response threshold ranged from 0.5 to 21.8 Hz and averaged 8.2 ± 1.1 Hz (Fig. 2). The mean heat-evoked change for OFF cells ranged over three orders of magnitude from −0.5 to −62.4 Hz and averaged −17.1 ± 2.9 Hz, corresponding to an average decrease of 171 spikes in the 10 s after tail heat onset. OFF cells with greater background discharge rates had larger heat-evoked decreases in discharge (Fig. 3). OFF cells were inhibited in an average of 97 ± 1% of the tail heat trials (Fig. 2). No OFF cell was excited by tail heat.

The heat-evoked changes in OFF cell discharge were significantly different from those observed in NEUTRAL cells that had an average heat-evoked decrease in discharge rate (t-test, P = 0.02). However, the tail heat-evoked changes in discharge in OFF and NEUTRAL cells were overlapping (Fig. 2).

The inhibition of OFF cells was characteristically indicated by a single ISI occurring after tail heat onset that was longer than the maximum baseline ISI. The maximum ISI recorded during tail heat stimulation averaged 41.9 ± 7.3 s compared with 0.9 ± 0.2 s during the baseline period before tail heat. The mean latency to the OFF cell pause averaged 3.2 ± 0.1 s (Fig. 2).
FIG. 4. Peak ON cell discharge rate evoked by tail heat was similar to the peak discharge rate recorded during background conditions. This resulted in a greater evoked change in discharge when the prestimulus discharge rate was low. A: background discharge of an ON cell, in the absence of any stimulation, is shown below the heart rate (top; scale on right) and blood pressure (middle; scale on left) traces. B: tail heat-evoked responses recorded from the same ON cell as shown in A. Top to bottom: heart rate (scale on right), blood pressure (scale on left), neuronal discharge rate, the rectified electromyographic (EMG) recording from the paraspinous muscles, and stimulus temperature. C: tail clamp-evoked responses recorded from the same ON cell as shown in A and B. After 2 trials, this cell was lost. Top to bottom: heart rate (scale on right), blood pressure (scale on left), neuronal discharge rate, the rectified EMG recording from the paraspinous muscles, and stimulus application. Time calibration, shown in A, is applicable to all panels.

Four OFF cells discharged rapidly (10–83 Hz) and regularly (CV_rate: 0.16–0.33) and did not contain bursting activity during background conditions. After the first noxious stimulus was applied, one of these four cells began to discharge irregularly (Fig. 2, \( \rightarrow \)). The remaining three cells continued discharging regularly and were inhibited for \(<1 \text{ h.} \)

This represented the first stimulus applied to the cell but not to the rat. Because of the protocol (see METHODS), a minimum of 15 min without stimulation preceded this first stimulus. However, the typical period without stimulation was closer to 1 h.
s by noxious heat; in contrast, all other OFF cells \((n = 27)\) were inhibited for \(>1\) s by noxious heat. Although the inhibition of these cells was brief, it was highly significant \((P < 0.0001)\) as it represented an increase of at least seven times SD\(_{IS}\) over background discharge values. For instance, in one case where the mean ISI during background conditions was 19 ms with a standard deviation of 4 ms, the mean pause evoked by tail heat was 49 ms.

**Neutral Cells.** Neutral cells \((n = 20)\) had no consistent response to noxious tail heat (Fig. 2). Neutral cells were excited (increased their discharge by \(\approx 2\) SD\(_{IS}\); see METHODS) by 16 \(\pm 4\)% of the trials and inhibited by 6 \(\pm 2\)% of the trials. The mean change in discharge evoked by tail heat was unrelated to the cell’s background discharge rate (Fig. 3).

**PSHT Cells.** Although eight p5HT cells were recorded, the discharge of only four p5HT cells was recorded in response to repeated tail heat trials. Two of these p5HT cells increased their discharge by 1 or 3 Hz in 100\% of the trials tested. The discharge of the remaining two p5HT cells was not affected by noxious heat.

**Other Cell Types.** Two cells exhibited increases in discharge in response to tail heat when their discharge was low before the trial onset and decreases in discharge when their discharge was high before the trial onset.\(^2\) The discharge of both of these cells varied in direct relation to the arterial blood pressure, which increased in trials that started when blood pressure was low and decreased in trials that started when blood pressure was high. In addition, one cell increased its discharge in response to noxious tail heat at a long latency \((10–20\) s). Because this neuron did not discharge before any of the tail heat trials, it may represent an OFF cell with a rebound burst discharge.

As mentioned earlier (see Background discharge patterns), 22 cells could not be classified because they discharged too infrequently to determine whether or not they were inhibited by noxious tail heat. These cells are likely to represent either OFF or NEUTRAL cells. An additional three cells could not be classified because there were too few trials that evoked either a motor withdrawal or a response.

### Responses to noxious and innocuous mechanical stimulation

No attempt was made to match the strength of the noxious mechanical and thermal stimuli, and the motor responses to these stimuli differed in their strength, muscle involvement, and timing. The movement evoked by noxious tail clamp was more vigorous than that evoked by heat and involved activation of hindlimb, trunk, and often forelimb musculature in addition to, and sometimes instead of, paraspinal muscles. The paraspinal muscle response, both visible and that revealed by the integrated EMG, to noxious tail clamp usually outlasted the stimulus and sometimes appeared to start after the stimulus offset (Figs. 4C, 5C, and 6).

Most ON cells \((22/23)\) were excited consistently by repeated trials of noxious tail clamp (Figs. 4C and 6B2). The remaining ON cell was not excited by tail clamp. Most ON cells \((15/17)\) also were excited by brief noxious pinches of both hindpaws and the tail, and no ON cell was inhibited by noxious pinch of any site tested. Six of 17 ON cells were excited by innocuous brushing of at least one site (both hindlimbs, the tail, and the trunk were tested in all cases; Fig. 7B3); no ON cells were inhibited by brush stimulation. When present, the responses to brush were usually shorter in duration and smaller in magnitude than were the responses to tail clamp or heat. Figure 7B illustrates the largest response to brush stimulation observed for any neuron in this study.

OFF cells \((17/17)\) were always inhibited by noxious tail clamp (Figs. 5C and 6A2). Similarly, all OFF cells tested \((11/11)\) were inhibited by brief noxious pinches of the hindpaws and tail. Innocuous brushing of the hindlimbs, tail and trunk inhibited 4 and excited 1 of the 11 OFF cells tested (Fig. 7A3). When present, the OFF cell responses to brush were always smaller in magnitude than were their responses to clamp or heat.

Noxious tail clamp evoked an excitatory \((n = 5)\) or inhibitory \((n = 3)\) response in 8 of 12 neutral cells tested (Fig. 6, C and D). One of 10 neutral cells tested was inhibited, and the remainder unaffected by brush stimulation.

### Relationship between neuronal and motor responses to noxious heat

Because the discharge of ON and OFF cells has been proposed to gate noxious-evoked withdrawal movements (Fields et al. 1983), ON and OFF cell responses to noxious tail heat were compared with evoked movements. The motor response to noxious tail heat was usually restricted to activation of paraspinal muscles that would lead to a flick of the tail (because the tail was taped down, no movement actually occurred). The mean duration of the early portion of the EMG response, which constituted the visible part of the motor response, was calculated from the averaged (rectified) EMG response recorded for each cell. The offset of this early EMG response occurred, on average, 8.7 \(\pm 0.3\) s after the stimulus onset (Fig. 8). The visible motor response outlasted the stimulus by \(>5\) s in only 3 of 67 cases. In some cases, a small motor response, which was only apparent in the rectified EMG trace was recorded after the stimulus temperature returned to innocuous levels (Fig. 8D, \(\rightarrow\)).

The mean latency of the tail flick withdrawal was not significantly different during recordings of ON \((3.46 \pm 0.11\) s) or OFF \((3.64 \pm 0.18\) s) cells \((t\text{-test}, P > 0.3)\). ON and OFF neuronal responses to noxious heat were sustained for tens of seconds to minutes and always outlasted the early portion of the motor response (see Fig. 8, A–E). For each cell, the latency at which discharge returned to baseline values was measured from the mean heat-evoked response.\(^4\) OFF cells returned to baseline discharge at a mean latency of 81 \(\pm 5\) s and ON cells returned to baseline discharge values at a mean latency of 62 \(\pm 4\) s. This difference was statistically significant \((t\text{-test}, P < 0.01)\).

\(^2\) A similar observation was reported for the responses of RM and NRM cells to sympathetic nerve stimulation (Blair and Evans 1991).

\(^3\) Because this cell did not discharge before any of the clamp trials, a possible inhibitory response cannot be ruled out.

\(^4\) A cutoff of 100 s was used in making these measurements.
FIG. 5. Neuronal discharge of an OFF cell in the absence of stimulation, in response to tail heat and clamp was recorded. A: background discharge in the absence of any stimulation. B: tail heat-evoked responses recorded from the same OFF cell as shown in A. Top to bottom: neuronal discharge rate, the rectified EMG recording from the paraspinous muscles, and stimulus temperature. C: tail clamp-evoked responses recorded from the same OFF cell as shown in A and B. Top to bottom: neuronal discharge rate, the rectified EMG recording from the paraspinous muscles, and stimulus application. Time calibration, shown in B, is applicable to all panels.

Response often had a similar time course as the second phase of the EMG activation (Fig. 8, D and F).

There were relatively few trials in which tail heat did not evoke a motor response. Such trials were examined to determine the relationship between ON or OFF cell responses and the occurrence of the motor response. In trials when no motor withdrawal occurred, ON cells were excited in only a minority of trials (8/17 trials; Fig. 9, B and C). In trials when no motor withdrawal occurred and an OFF cell was active (≥1 Hz) before the stimulus application, noxious heat stimulation always evoked an inhibitory response in OFF cells (13/13 trials; Fig. 9A). The proportion of ON cell responses that occurred in trials that failed to elicit a motor withdrawal was significantly less than for OFF cells (χ² test, P = 0.002). To further examine the association or dissociation between the neuronal and motor responses, trials in which a motor response, but no neuronal response, occurred were examined. ON and OFF cells failed to respond in 27/227 and 3/106, respectively, of all trials that evoked a motor withdrawal. This difference was statistically significant (χ² test, P = 0.01).

Evoked responses to noxious heat usually but not always began before the onset of the motor response (Fig. 10). The onsets of the OFF cell pause and ON cell burst averaged 680 ± 180 ms and 420 ± 90 ms before the tail flick onset, respectively. There was a wide range of response latencies in individual trials, ranging from 2.6 s before to 1.8 s after the onset of the motor withdrawal. The neuronal response began after the tail flick onset, at least once, in 10/28 OFF and 25/49 ON cells (Fig. 10, A and C, n and c, under the line). For six OFF cells and eight ON cells, the average response latency was greater than the average tail flick latency (Fig. 10, D, n and c, under the line).

For all cells that responded to noxious stimulation, time histograms were constructed using either the time of the stimulus onset or the time of tail flick withdrawal as zero (Fig. 11, A and B). The first derivative of the smoothed histograms was used to calculate the maximum and minimum slopes. For ON cells, the ratio of the maximal slope of the flick-aligned histogram to that of the heat-aligned histogram was calculated (Rmax). Similarly, for OFF cells, the ratio between the minimal slopes was calculated (Rmin). In the case of both ON and OFF cells, there were individual neurons that showed a more rapid change in discharge in relation to the flick than in relation to the heat onset as well as those that showed a more rapid change in discharge in
FIG. 6. Tail clamp (A2±D2) evoked a similar neuronal response as tail heat (A1±D1) in ON (B, 1 and 2) and OFF (A, 1 and 2) cells but not in NEUTRAL cells (C1±D2). For each of the 4 cells illustrated, poststimulus time histograms are shown for both tail heat (left) and tail clamp (right) trials. In each panel, the traces, from top to bottom, are the mean discharge rate (100 ms bins), the averaged rectified EMG, and the averaged stimulus (stimulus temperature in A1±D1 and tail clamp application in A2±D2). Tail clamp (A2 and B2) elicited a response that was similar to that evoked by tail heat (A1 and B1) in most ON (B) and OFF (A) cells. Although they did not respond to tail heat (C1 and D1), some NEUTRAL cells (C and D) responded to noxious tail clamp (C2 and D2). The number of trials averaged is 5 in all panels except B1 and D1 in which 6 trials are averaged. Time calibration, shown in A1, is applicable to all panels.

As shown in Table 1, the response of ON and OFF cells was not related to the presence of an autonomic response. The autonomic responses evoked by tail heat were very consistent, occurring in >90% of the tail heat trials. In the rare trials in which changes for 62% of the OFF cells and 45% of the ON cells, the response slopes measured from histograms aligned to the flick or to the heat were equal or within 25% of equal (Fig. 11C).

Relationship between neuronal and autonomic responses to noxious heat

The baseline blood pressure (OFF cells: 119 ± 4 mmHg; ON cells: 117 ± 2 mmHg) and heart rate (OFF cells: 345 ± 9 bpm; ON cells: 336 ± 6 bpm), measured before tail heat stimulation, were not different when recording ON or OFF cells. In response to noxious tail heat, heart rate nearly always increased (41/42 cells) by an average of 15 ± 1 bpm. The blood pressure response to noxious tail heat was more variable and included both pressor (n = 25 cells; mean: 11 ± 1 mmHg) and depressor (n = 17 cells; mean: −10 ± 1 mmHg) responses.
FIG. 7. Brush stimulation (A3 and B3) elicited a smaller response than that evoked by either tail heat (A1 and B1) or tail clamp (A2 and B2) in ON (B) and OFF (A) cells. Responses to tail heat and tail clamp (stimuli are shown bottom traces) are poststimulus time histograms (100-ms bins) averaged from 5 trials. Responses to brush are shown as histograms (100-ms bins). For brush stimulation, single trials were used and the stimulus site is listed below the stimulus trace. Time calibration, shown in A1, is applicable to all panels.

the nucleus of the trapezoid body, inclusive (Fig. 13). ON, OFF, and NEUTRAL cells were not preferentially distributed between these nuclei. p5HT cells were contained in either RM (n = 5) or in the ventral portion of NRMC, termed NRMCa (n = 2) (Newman 1985a,b). The locations of 15 cells, including 8 ON, 1 OFF, 1 NEUTRAL, 1 p5HT, 3 unclassified, and 1 “other” type cells could not be determined.

DISCUSSION

Quantitative method for classifying RM and NRMC neurons

In the anesthetized rat, the spontaneous discharge of most RM cells is irregular and contains bursts and pauses that resemble noxious stimulus-evoked responses. A true “response” to noxious stimulation can only be distinguished from a naturally occurring change in discharge by a quantitative comparison between background and stimulus-evoked discharge patterns. Such a comparison reveals that the mean responses of ON and OFF cells are more than four times greater than the average change in rate that is observed spontaneously. Furthermore, as summarized in Fig. 3, these responses are elicited consistently by repeated trials of noxious stimulation.

Because it does not depend on the idiosyncrasies of nociceptive testing, the classification system presented here can be used in other laboratories. The requirements are that a period of background discharge, in the absence of stimulation, is recorded and analyzed; the nociceptive stimulus reaches noxious thermal levels; each stimulus application is of standard duration and magnitude regardless of the motor response elicited; and a motor response is elicited. The timing of the “response period” for analysis can be adjusted to the particular stimulus used such that it includes the period during which most motor withdrawals are elicited.

Distribution and frequency of cell types in the RM and NRMC

In an attempt to completely survey all physiological cell types present in the RM and NRMC of the anesthetized rat, units were recorded solely based on isolation properties in the present study. Among nonserotonergic cells, the number of ON cells was greater than that of OFF cells, a finding that is in agreement with several previous studies in acutely anesthetized preparations (Chiang and Gao 1986; Fields et al. 1983; Guilbaud et al. 1980; Morgan and Fields 1994; Morgan and Heinricher 1992; Rosenfeld et al. 1990; Thurstson and Randich 1992). The proportions of OFF (23%) and NEUTRAL (16%) cells observed were likely to be slightly
Neuronal response to noxious tail heat persisted beyond the early motor response in the case of ON (A and B) and OFF cells (C and D). This relationship held for all ON and OFF cells tested (E). Offset of a late motor response (D, →), which was only present in some cases, occurred close to the offset of the neuronal response (F). In A–D, the traces, from top to bottom, are the mean discharge rate (100-ms bins), the averaged rectified EMG, and the averaged stimulus temperature. Neuronal responses to tail heat are poststimulus time histograms averaged from 5 trials. Time calibration shown in C is applicable to A–D. E and F: latency to the offset of the neuronal response (measured from the poststimulus time histograms) is compared with the latency to the offset of the early (E) or late (F) motor response (measured from the mean rectified EMG) for each cell (symbols as in F). Offset latencies ≥ 100 s were estimated at 100 s. Mean values for all ON and OFF cells are shown by △ and ▽. The unity line (y = x) is presented on each graph.
underestimated compared with other studies because ~20% of the RM/NRMC cells recorded could not be classified but likely belonged to one of these two cell classes. Intermediate cell types, such as cells with biphasic or long latency responses, were rarely observed. Thus it is likely that the original three cell classes described by Fields et al. (1983) adequately describe the major nonserotonergic physiological cell types present in the RM and NRMC.

**Classification of nonserotonergic regularly discharging neurons**

In previous reports from this and other laboratories, cells with fast and regular discharge have been grouped together into a distinct cell class and termed "regular" cells (Gao et al. 1997; Leung and Mason 1995, 1996; Oliveras et al. 1989). This classification was based on initial findings that these cells had a distinctive discharge pattern, were opioid-insensitive, and were unaffected or weakly excited by noxious stimulation. The distinctive characteristics of these neurons suggested a physiological function different from that of irregularly firing neurons.

In the current study, we have chosen to classify regularly discharging neurons as ON, OFF, and NEUTRAL cells because the additional data reported here indicate that these neurons do not exhibit an homogeneous response to noxious stimulation. The noxious heat-evoked responses of unaffected and excited regularly discharging neurons were indistinguishable from those of NEUTRAL and ON cells, respectively. In addition, an important role for regular background discharge in nonserotonergic modes of nociceptive modulation has not been demonstrated. It is also not clear that a regular discharge pattern always, or even usually, persists in the unanesthetized state (Oliveras et al. 1991a,b; unpublished observations).

**Limitations of this classification system**

There are two limitations to the cell-classification system presented here. First, spontaneously inactive neurons were not studied. The second limitation is that this classification system is only directly applicable to the isoflurane-anesthetized condition. However, it is unlikely that the cell-class properties described here are dependent on the type or presence of anesthesia as similar physiological properties are observed in RM and NRMC cells recorded under halothane (Mason et al. 1990; Morgan and Heinricher 1992), ketamine (McGarvaughy and Reinis 1993; McGaraughty et al. 1993), and barbiturate (Fields et al. 1983) anesthesia as well as under decerebrate conditions (Clarke et al. 1994). Although it is possible that other types of noxious stimulus-evoked responses occur in the unanesthetized condition, none have been described that have not been observed in the anesthetized condition (Fornal et al. 1985; McGaraughty et al. 1993; Oliveras et al. 1989, 1990, 1991a,b; unpublished observations). Specifically, the four RM and NRMC cell types described above—ON, OFF, NEUTRAL, and p5HT—have been
FIG. 10. Relationship between the latencies of ON and OFF cell responses and of the tail flick withdrawal. A: latency of the OFF cell pause is compared with the tail flick latency for all trials where the rat withdrew and the OFF cell responded. B: length of the OFF cell pause is compared with the tail flick latency for all trials where the rat withdrew and the OFF cell responded. C: latency to the ON cell burst is compared with the tail flick latency for all trials where the rat withdrew and the ON cell responded. D: mean latency of the tail flick withdrawal is compared with the mean latency of the cellular response for ON and OFF cells. Line $y = x$ is presented in A, C, and D.

observed in the unanesthetized as well as anesthetized condition.

Functional roles of ON and OFF cells

Based on their nocifensor reflex-related activity, OFF cells originally were hypothesized to gate spinal nociceptive transmission in a manner analogous to the gating of saccadic eye movements by omnipause neurons (Fields et al. 1983). That is, the inhibition of OFF cell discharge evoked by a noxious stimulus was thought to be necessary to disinhibit dorsal horn neurons, thereby permitting nociceptive transmission and the execution of nocifensor reflexes. The evidence for this idea included the findings that the OFF cell pause evoked by noxious tail heat occurred before the motor response and that the OFF cell inhibition was more rapid when aligned to the motor response than to the stimulus onset. In contrast, we observed that OFF cells often ceased discharging after the tail flick onset. Furthermore, the mean flick to heat slope ratio for OFF cells was distributed unimodally about a mean of 1.0. Our finding that OFF cells responded similarly to tail heat regardless of whether the stimulus elicited a tail flick response is also evidence that the OFF cell pause can be dissociated from the initiation of the motor response. Although differences in anesthetic agent, anesthetic depth, and the rise time of the tail heat stimulus may contribute to the differences between the present and previous findings, our results do not support a premotor function, analogous to that of the omnipause neuron, for OFF cells.

The spontaneous discharge of most ON and OFF cells contained bursts and pauses. Because these bursts and pauses occurred in the absence of noxious stimulation, inputs other than noxious tail heat or clamp must influence the discharge of RM ON and OFF cells. We previously have demonstrated that the bursts of ON and OFF cells occur during local de-
creases or increases, respectively, in blood pressure (Leung and Mason 1996). Thus signals related to cardiovascular activity are one nonnociceptive source of RM input. Furthermore, there is evidence that RM ON and OFF cells respond to innocuous somatosensory and auditory stimulation in the unanesthetized condition (Oliveras et al. 1989, 1990; unpublished observations). Future experiments are needed to establish whether additional sources of nonsomatic input, such as vestibular- or respiratory-related signals, influence RM cell discharge. Even with our present knowledge, it is clear that nonnoxious inputs strongly influence RM ON and OFF cell discharge. Although the pain modulatory field has focused on pain’s modulation of pain, these findings suggest that ON and OFF cells modulate nociceptive transmission in reaction to a number of physiological inputs, nonnociceptive as well as nociceptive.

The modulatory effects of ON and OFF cells are likely to be concentrated in the seconds and minutes after the stimulus occurrence. The bulk of the change in ON and OFF cell discharge evoked by tail heat occurs after the onset of the motor response and even after the conclusion of the visible motor response. At this time, motoneuron and muscle activity remain elevated although visible movements are no longer evident. ON and OFF cells may affect spinal processes during

![Graph showing poststimulus time histograms](image)

**TABLE 1.** Number of trials in which ON and OFF cells responded to tail heat and there was a change in heart rate or blood pressure

<table>
<thead>
<tr>
<th></th>
<th>OFF Cells</th>
<th>ON Cells</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Response</td>
<td>Response</td>
<td>No Response</td>
<td>Response</td>
</tr>
<tr>
<td>Heart rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No response</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Response</td>
<td>2</td>
<td>64</td>
<td>15</td>
<td>100</td>
</tr>
<tr>
<td>Blood pressure</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No response</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>8</td>
</tr>
<tr>
<td>Response</td>
<td>32</td>
<td>68</td>
<td>15</td>
<td>96</td>
</tr>
</tbody>
</table>
FIG. 12. Tail heat-evoked responses of ON and OFF cells were independent of the evoked changes in heart rate and blood pressure. Top to bottom: heart rate (scale on right), blood pressure (scale on left), neuronal discharge rate, the rectified EMG recording from the paraspinal muscles, and stimulus temperature. A: OFF cell that failed to respond to a tail heat trial (dashed arrow) that elicited a response in heart rate and blood pressure. B: ON cell that failed to respond to a tail heat trial (dashed arrow) that elicited a response in heart rate and blood pressure. In contrast, this ON cell responded to a tail heat trial that failed to elicit a response in blood pressure (small arrow).

this prolonged motor response, which occurs after the initial rapid withdrawal. The idea that ON and OFF cell modulation targets movements after their initiation is supported by the finding that electrical stimulation of tooth pulp evokes synaptic responses in RM and NRMC cells that occur after the onset of the reflex withdrawal (Mason et al. 1986). Furthermore, Ramirez and Vanegas (1989) reported an example of phasic modulation in which tooth pulp stimulation shortened
the withdrawal latency evoked by a subsequent tail heat stimulus. In summary, it is possible that ON and OFF cells play an important role in modulating the late responses to noxious stimulation as well as the responses to subsequent noxious insults.

**Function of NEUTRAL cells**

The lack of NEUTRAL cell responses to noxious thermal stimulation and opioid administration does not preclude a role for these cells in nociceptive modulation. It is possible that NEUTRAL cells are only affected by more robust stimuli or motor responses or by nonthermal modalities of noxious stimulation. In support of these ideas, 67% of the NEUTRAL cells, defined by their unresponsiveness to noxious heat, responded to noxious clamp of the tail with either an increase or a decrease in discharge. NEUTRAL cells then may represent ON or OFF cells that only respond to particular intensities and/or modalities of painful stimuli. Future experiments are required to examine this issue.

**RM/NRMC and autonomic status**

Previous reports (Leung and Mason 1996; Thurston and Randich 1992) have established that ON cells typically increase their discharge during periods of low blood pressure, whereas OFF cells burst during periods of high blood pressure. Because tail heat evokes changes in blood pressure and heart rate, it is possible that the changes in ON and OFF cell discharge evoked by tail heat are secondary to an autonomic response. This is unlikely, however, because the neuronal response to noxious heat occurred in trials that did not elicit a cardiovascular response. In addition, the responses of ON and OFF cells were similar regardless of whether tail heat produced a pressor or a depressor effect. Finally, the failure of ON and OFF cells to respond to noxious tail heat was never accompanied by the absence of a cardiovascular response.

**Summary**

The original cell classes described by Fields and colleagues—ON, OFF, and NEUTRAL—are inclusive of the great majority of nonserotonergic cell types encountered in RM and NRMC. Serotonergic cells comprise the only necessary additional cell class. The assignation of RM and NRMC nonserotonergic cells to the ON, OFF, and NEUTRAL cell classes is best accomplished by a quantitative comparison of heat-evoked responses to the spontaneous variability present in discharge during periods without stimulation. The consistency, not the magnitude, of responses to repeated and standard noxious thermal stimulation distinguished ON and OFF from NEUTRAL cells.

The noxious stimulus-evoked responses of ON and OFF cells were typically sustained beyond the period of the visible motor withdrawal. It is therefore likely that the principal modulatory effect of ON and OFF cells focuses on the preparation for subsequent movements in the moments after an insult rather than on the initiation of the first withdrawal. Because the initial withdrawal from noxious stimulation prevents injury from potentially tissue-damaging stimuli, it is not surprising that putative nociceptive modulatory neurons such as ON and OFF cells may leave this initial response relatively unchanged. However, inasmuch as a noxious event is a strong predictor of subsequent noxious events, modulatory action in the seconds and minutes after an initial insult may be advantageous. At this time, the most protective reaction to persistent or recurrent noxious stimulation is likely to be more complex than a simple withdrawal. For instance, after withdrawing one’s foot from the teeth of a predator, flight (running away), fight (attacking the predator), or freezing (playing dead) are likely to be more protective strategies than simply continued flexor withdrawal. Changes in RM and NRMC cell discharge may influence these complex reactions via modulation of nociceptive processing, autonomic regulation and/or motor tone.

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