Serotonergic Raphe Magnus Cells That Respond to Noxious Tail Heat Are Not ON or OFF Cells

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Received 11 April 2000; accepted in final form 19 June 2000

Gao, Keming and Peggy Mason. Serotonergic raphe magnus cells that respond to noxious tail heat are not on or off cells. J Neurophysiol 84: 1719–1725, 2000. Pharmacological studies have suggested that serotonergic cells in RM contribute to both the inhibition and facilitation of spinal nociceptive transmission. Physiological studies in the medullary nucleus raphe magnus (RM) and adjacent nucleus reticularis magnocellularis have identified putative nociceptive-inhibitory off cells and nociceptive-facilitatory neurons on cells by their responses to noxious thermal stimulation. The present study was designed to determine 1) whether any serotonergic RM cells respond to noxious thermal stimulation and 2) whether noxious heat-responsive serotonergic cells should be classified as ON or OFF cells. Serotonergic cells (n = 150) were identified by physiological criteria in anesthetized rats; 30 of 32 cells tested contained serotonin immunoreactivity. Noxious tail heat elicited a neuronal response in less than a quarter of the serotonergic cells. Most serotonergic cells that responded to tail heat were excited (n = 25), while a small minority of the cells tested were inhibited (n = 8). The tail heat-evoked responses of serotonergic cells were small in magnitude, averaging five to eight spikes in 10 s. Excitatory responses rarely persisted for more than 10 s, while inhibitory responses rarely persisted for more than 20 s. The tail heat-evoked responses of serotonergic cells were compared to those of non-serotonergic cells (n = 186). Non-serotonergic cells that responded to noxious tail heat had significantly greater response magnitudes, averaging 75–95 spikes in 10 s, than heat-responsive serotonergic cells. In addition, most heat-responsive non-serotonergic cells responded for at least 30 s after stimulus onset. These results demonstrate that the tail heat-evoked responses of serotonergic RM cells are qualitatively and quantitatively distinct from those of non-serotonergic ON and OFF cells. It is therefore unlikely that serotonergic RM cells, even the subpopulation that responds to noxious tail heat, share a physiological function with ON and OFF cells.

INTRODUCTION

Neurons in the medullary raphe magnus (RM) and adjacent nucleus reticularis magnocellularis (NRMC) project to the superficial spinal dorsal horn and are thought to participate in the modulation of spinal nociceptive transmission (Basbaum et al. 1978; Fields et al. 1991; Mason 1999). Electrical stimulation of the RM and NRMC either suppresses or facilitates noxious heat-evoked motor and neuronal responses (Zhao and Gebhart 1990, 1992, 1997). Inactivation of the RM and NRMC attenuates both opioid analgesia and the hyperalgesia associated with naloxone-precipitated opioid withdrawal (Deakin and Dostrovsky 1978; Kaplan and Fields 1991). These findings support the idea that at least two different cell types are present in RM: one cell type that suppresses and one that facilitates nociceptive transmission. Physiological studies provide evidence that a RM cell’s response to noxious thermal stimulation predicts both its response to opioids and its physiological function (Barbaro et al. 1986; Bederson et al. 1990). Specifically, OFF cells are inhibited by noxious stimulation, excited by opioids and likely suppress nociceptive transmission, while ON cells are excited by noxious stimulation, inhibited by opioids and may facilitate nociceptive transmission (Fields et al. 1983a, 1991). A third cell type, the neutral cell, is unaffected by noxious tail heat or opioids, and its role in nociceptive modulation is unclear.

To understand how ON, OFF, and neutral cells modulate nociceptive transmission, it is critical to know which neurotransmitters are contained within these cells. One obvious possibility is that all or some of the cells in each of these classes contains serotonin. Serotonergic neurons in RM and NRMC are the major source of serotonin in the spinal and medullary dorsal horns (Dahlstrom and Fuxe 1964; Johansson et al. 1981; Oliveras et al. 1977). Iontophoretic or local spinal administration of serotonin receptor antagonists attenuates the antinociceptive effects of RM activation (Barbaro et al. 1985; Hammond and Yaksh 1984; Jensen and Yaksh 1986; Vasko et al. 1984). Intrathecal administration of methysergide, a serotonin receptor antagonist, also attenuates the facilitation of nociceptive transmission evoked by NRMC activation (Zhao and Gebhart 1991). These data implicate serotonergic RM and NRMC neurons in both the facilitation and the suppression of nociceptive transmission and suggest that at least some ON and OFF cells may contain serotonin.

Serotonergic RM cells differ from their non-serotonergic neighbors by having a slow and steady discharge, a small dendritic arbor, and a distinctive axonal morphology (Gao and Mason 1997; Mason 1997). Yet, it remains unclear whether serotonergic and non-serotonergic cells differ in their responses to noxious tail heat. This is an important issue because, as described above, the response to noxious tail heat is predictive of a RM cell’s response to opioids and its physiological function. Initial reports were that neither ON (0/8) nor OFF (0/9) cells contain serotonin (Potrebic et al. 1994), but subsequent studies provide evidence that at least some of these cells do contain serotonin (Deakin and Dostrovsky 1978; Kaplan and Fields 1991). These findings support the idea that at least two different cell types are present in RM: one cell type that suppresses and one that facilitates nociceptive transmission. Physiological studies provide evidence that a RM cell’s response to noxious thermal stimulation predicts both its response to opioids and its physiological function (Barbaro et al. 1986; Bederson et al. 1990). Specifically, OFF cells are inhibited by noxious stimulation, excited by opioids and likely suppress nociceptive transmission, while ON cells are excited by noxious stimulation, inhibited by opioids and may facilitate nociceptive transmission (Fields et al. 1983a, 1991). A third cell type, the neutral cell, is unaffected by noxious tail heat or opioids, and its role in nociceptive modulation is unclear.
studies reported that a minority of serotonergic cells (5/13) were either excited or inhibited by noxious tail heat (Gao et al. 1997, 1998; Mason 1997). These conflicting reports likely stem from the small and biased samples studied and the nonquantitative methods used for evaluating the responses to noxious heat. Recently, we described a novel quantitative method for classifying the responses of RM neurons to noxious tail heat (Leung and Mason 1998). This method was used to quantitatively characterize the responses to noxious tail heat of a large sample of serotonergic cells, to compare these responses to those of non-serotonergic cells, and thereby to determine whether any serotonergic cells comprise a subset of ON and/or OFF cells.

METHODS

Surgical preparation

Male Sprague-Dawley rats (240–400 g; Sasco, Madison, WI) were deeply anesthetized with 1.8–2.0% halothane in oxygen via a tracheal catheter. A small craniotomy was made for the introduction of recording microelectrodes. Electromyographic (EMG) electrodes were placed transcutaneously in the paraspinous muscles to record motor withdrawal from noxious tail heat. Core body temperature of all rats was maintained at 37–38°C by use of a water-perfused heating pad.

Protocol

When the surgical preparation was complete, the anesthetic concentration was decreased to 1.0% halothane. This anesthetic concentration produced a light plane of anesthesia at which the rats were able to withdraw from a noxious stimulus but showed no gross purposeful movement in the absence of noxious stimulation. After equilibration, a glass micropipette was introduced into the region of the RM and NRMC (1.5–2.6 mm from interaural zero, L 0–1.0 mm, V 9.0–10.5 mm from the cerebellar surface). A spontaneously active unit was isolated and its background activity recorded for 5–15 min in the absence of stimulation and at a steady-state concentration of halothane.

The responses of cells to repeated (n = 3–5) noxious tail heat applications were recorded. The heat stimulus was applied, using a pellet device (TDI, Minneapolis, MN), to a 2.5-cm patch of the tail that was centered 6–7 cm from the tip. Each tail heat stimulus consisted of a 2-s ramp that increased the pellet temperature from 32 to 56°C followed by a 4-s plateau at 56°C. The tail was taped to the pellet platform and therefore was exposed to the full duration stimulus in every trial. Heat stimuli were applied at intervals of 4 min.

Neurons that remained after the complete physiological protocol were intracellularly labeled with Neurobiotin, which was subsequently visualized with a Texas Red fluorophore. These cells were then processed for serotonin immunofluorescence, using a Bodipy fluorophore, as previously described (Mason 1997). In the case of cells that were not successfully labeled, the recording site was marked by iontophoresis Neurobiotin into the extracellular space and subsequently visualized with 3,3′diaminobenzidine (Gao and Mason 1997).

All p5HT cells were studied. The nonserotonergic cells that are included in this report were recorded as part of a number of other studies on-going in the lab. For several of these experiments, we were particularly interested in either ON or OFF, but not NEUTRAL, cells. Therefore the proportions of excited, inhibited, and unaffected non-p5HT cells in the present manuscript are likely to less accurately represent the RM non-p5HT cell population than the proportions reported in Leung and Mason (1998), a study that lacked any intentional sampling bias.

Data were acquired onto a Pentium III attached to a Micro1401 A/D converter (CED, Cambridge, UK). The unit was acquired at 20 kHz, and the EMG and stimulus temperature at 1 kHz. Activity from single units was discriminated off-line (Spike3, CED, Cambridge, UK). All experiments were also acquired onto a VCR recorder (Vetter Instruments, Rebersburg, PA) for backup.

Analysis

CELL CLASSIFICATION. The mean (x), standard deviation (SD), and coefficient of variation of the interspike interval (CV) were calculated from the recording of background activity (see above). For each cell, the value of the function, y(x, SD) = 146 – x + 0.98 SD, was calculated, where x and SD are in ms. Cells were classified as p5HT if the function value was less than zero and as non-p5HT if the function was greater than zero (Mason 1997). All cells were further characterized by their response to tail heat as described previously (Leung and Mason 1998). Briefly, the variability in background discharge was first quantified as the standard deviation of the change across 10-s bins normalized to impulses/second (SD10 s) (Leung and Mason 1998). Unit responses to peltier heat stimulation were then calculated as the difference in unit firing rates (in impulses/s) before and after stimulus application (10 s before and for sequential 10-s periods starting 2 s after stimulus onset). Heat-evoked increases in discharge that were greater than two SD10 s, were considered excitatory responses, and evoked decreases that were greater than two SD10 s were considered inhibitory responses. This method allows us to have confidence, at a P < 0.05 level, that the change evoked by a single stimulation trial is unlikely to have occurred spontaneously.

The percentage of excitatory and inhibitory responses to tail heat stimulation were calculated for each cell. Cells that were excited by a majority of heat applications were considered to be excited by tail heat. Cells that were inhibited by a majority of heat applications were considered to be inhibited by tail heat. Cells that did not respond to a majority of the trials were considered to be unaffected. Note that by establishing these criteria, the probability that a set of responses would occur by chance is vanishingly small. For example, the probability of a cell responding to four of five heat trials by chance would be 7 × 10–4.

RESPONSE DURATION. In a previous report describing the duration of ON and OFF cell responses to noxious tail heat, we determined the time point at which discharge returned to baseline values (Leung and Mason 1998). However, this procedure is not accurate for slowly firing neurons such as p5HT cells. For instance, it would be difficult to assign a response offset to cells such as some of those illustrated in Fig. 6. Therefore we have analyzed each cell’s responses during four post-stimulus 10-s periods. Cells that respond in the first period but not in the second period are assigned a duration of 10 s. Cells that respond in the first and second periods but not in the third period are assigned a duration of 20 s. Similarly, cells that respond in the first, second, and third periods and those that respond during all four periods are assigned durations of 30 and 40 s, respectively.

STATISTICS. Statistical analyses were performed using SAS (Cary, NC) and Microsoft Excel 5.0 (Redmond, WA). All values are reported as means ± SE.

RESULTS

Characterization of serotonergic cells

A total of 152 p5HT neurons were identified by their slow and steady background discharge (see METHODS) (Mason 1997). Of the 32 intracellularly labeled p5HT cells, 30 contained serotonin immunoreactivity (Fig. 1). The two p5HT cells that did not contain serotonin immunoreactivity have not been included in the sample that was analyzed and described below. All p5HT cells were located in RM or NRMCα throughout the
rostrocaudal extent of the facial nucleus (Fig. 2). The mean rate of discharge of the studied cells was 1.6 ± 0.1 (SE) Hz and the mean CV ISI of p5HT cells was 0.45 ± 0.01 (Fig. 3, Table 1).

Serotonergic cell responses to noxious tail heat

Most p5HT neurons (114/150; 76%) did not respond to noxious tail heat. In non-responsive neurons, heat evoked an average change in discharge of only 0.6 ± 0.2 spikes in 10 s.

A minority of p5HT neurons were excited (25/150; 17%) by noxious heat (Fig. 4A). Among these cells, the mean increase in cell discharge evoked by tail heat was 7.9 ± 1.6 spikes during the initial 10 s after heat onset. Most (20/25) p5HT cells were excited by tail heat for a total duration of 10 s, and only 1 of 25 cells was excited for the entire 40-s period analyzed (t-test for proportions, P < 0.001; Fig. 5). The mean total response magnitude (total number of spikes during all responsive periods) was 10.7 ± 2.9 spikes.

Heat-excited p5HT cells were excited by 88 ± 3% of the noxious tail heat trials and were never inhibited by tail heat. The slope of the onset of the excitation for individual cells was not different when discharge was aligned to the heat stimulus or to the motor withdrawal (Fig. 6A).

Eight of 150 (5%) p5HT neurons tested were inhibited by noxious heat (Fig. 4B). Among these cells, the mean decrease in cell discharge evoked by tail heat was 5.4 ± 1.1 spikes in 10 s. The mean total response was a decrease of 11.6 ± 5.4 spikes. The discharge of half of the heat-inhibited cells remained below baseline levels for 20 s, but only one of the eight cells was inhibited for longer than 20 s (Fig. 5). Heat-inhibited

| TABLE 1. Mean rate of discharge and CV ISI of the cells studied |
|------------------|----------------|----------------|
| Cell Type         | n  | Rate, Hz | CV ISI |
| Serotonergic      |    |          |        |
| Excited by TH     | 25 | 1.5 ± 0.1 | 0.43 ± 0.03 |
| Inhibited by TH   | 8  | 1.5 ± 0.1 | 0.38 ± 0.04 |
| Unaffected by TH  | 114| 1.6 ± 0.1 | 0.46 ± 0.01 |
| All               | 150| 1.6 ± 0.1 | 0.45 ± 0.01 |
| Non-serotonergic  |    |          |        |
| Excited by TH     | 52 | 9.5 ± 1.3 | 1.91 ± 0.39 |
| Inhibited by TH   | 75 | 16.2 ± 1.4| 0.67 ± 0.08 |
| Unaffected by TH  | 44 | 13.3 ± 1.6| 0.71 ± 0.08 |
| All               | 184| 13.0 ± 0.8| 1.07 ± 0.12 |

Values are means ± SE; n is number of cells. Two non-p5HT cells that were excited by tail heat but were not spontaneously active are not included in this table. TH, tail heat.
p5HT cells were inhibited by $74 \pm 7\%$ of the noxious tail heat trials and were never excited by tail heat. Given the low discharge rate of the p5HT cells, it is not surprising that a sharp onset to the inhibition was not obvious even in records averaged from multiple trials. This was true regardless of whether discharge was aligned to the heat stimulus or to the motor withdrawal (Fig. 6B).

Three p5HT cells were inconsistently affected (e.g., 1 excitation, 1 inhibition, and 1 no effect) by tail heat, and their responses were not classified.

The CV ISI s and mean background ISIs of cells that were excited, inhibited, or unaffected by tail heat were not different (Fig. 3, Table 1).

Characterization of non-serotonergic cells

Non-p5HT cells ($n = 186$) were located in RM or NRMC (not shown). These cells were distinguished from p5HT cells by a higher rate of discharge and/or a more irregular discharge (Fig. 3; Table 1). A subset of non-p5HT cells ($n = 22$) was intracellularly labeled and tested for serotonin immunoreactivity. None of the non-p5HT cells tested contained serotonin immunoreactivity.

Non-serotonergic cell responses to noxious tail heat

Non-p5HT cells that were excited by noxious tail heat ($n = 54$) responded with a mean increase in cell discharge of 75.5 ± 11.0 spikes in 10 s (Fig. 4C). These cells were excited by $87 \pm 2\%$ of the noxious tail heat trials and were not inhibited by tail heat. Most of the heat-excited non-p5HT cells were excited for at least 30 s (Fig. 5). The total response tended to be greater for cells that were excited for longer durations (Fig. 5). The mean total response for all non-p5HT cells that were recorded for 40 s poststimulus was 158.8 ± 24.5 spikes. It should be noted that this is likely to be an underestimate as some cells continued to respond beyond 40 s.

The mean decrease in discharge evoked by tail heat among non-p5HT cells that were inhibited ($n = 75$) was 93.4 ± 9.1 spikes in 10 s (Fig. 4D). The discharge of most of these cells remained inhibited for the entire response period analyzed, resulting in a mean total response of 282.2 ± 44.0 spikes (Fig. 5). As above, the total response of cells that were inhibited for a longer duration was both likely to be an underestimate and tended to be greater than that of cells that were inhibited for only 10–20 s (Fig. 5). Heat-inhibited non-p5HT cells were inhibited by $86 \pm 2\%$ of the noxious tail heat trials and were not excited by tail heat.

The remaining non-p5HT cells were either unaffected by tail heat ($n = 44$) or could not be classified ($n = 13$). The latter group consisted of cells that discharged too infrequently to...
determine whether or not they were inhibited by noxious tail heat \((n = 10)\) and cells for whom there were too few heat trials that evoked a motor withdrawal \((n = 3)\) (Leung and Mason 1998).

**Comparison of serotonergic and non-serotonergic cell responses to tail heat**

The changes in discharge evoked by noxious tail heat were compared (ANOVA, Tukey’s) between the three subgroups (heat-excited, heat-inhibited, and heat-unaffected) of p5HT and non-p5HT cells. Both the initial \((2–12 \text{ s})\) and the total heat-evoked decreases in discharge were significantly greater for heat-inhibited non-p5HT cells than for heat-inhibited p5HT cells \((P < 0.001)\). The initial heat-evoked increase in discharge was significantly greater for heat-excited non-p5HT cells than for heat-excited p5HT cells \((P < 0.0001)\). In contrast, there was no difference between the heat-evoked responses of heat-unaffected p5HT and non-p5HT cells.

The duration of the response to noxious heat was shorter for heat-responsive p5HT cells than for heat-responsive non-p5HT cells (Figs. 4 and 5). Among heat-excited cells, p5HT neurons were rarely excited for more than 10 s \((5/25)\), while most non-p5HT cells \((39/54)\) were excited for at least 20 s \((t\text{-test for proportions}, P < 0.001)\). Similarly, the proportion of heat-inhibited p5HT cells \((1/8)\) that were inhibited for more than 20 s was significantly less than the proportion of heat-inhibited non-p5HT cells \((51/75)\) that were inhibited for more than 20 s \((t\text{-test for proportions}, P = 0.002)\).

**Discussion**

There are several important differences between the tail heat-evoked responses of serotonergic and non-serotonergic RM cells. First, the discharge of more than 75% of serotonergic but less than half of non-serotonergic RM neurons is unaffected by noxious tail heat (Leung and Mason 1998). Second, when present, the responses of heat-responsive serotonergic RM cells are significantly smaller in magnitude and shorter in duration than those of non-serotonergic cells. These results, obtained in the present study, suggest that non-serotonergic but not serotonergic cells are well suited to the modulation of nocifensive responses in the seconds and minutes after acute noxious stimulation. Finally, the response of non-serotonergic, but not serotonergic, cells to noxious tail heat predicts the response to opioids (Barbaro et al. 1986; Cheng et al. 1986; Gao et al. 1998). These results demonstrate that the serotonergic RM cells are not likely to play a role in phasic nociceptive modulation. Instead, the nociceptive modulatory neurons that are involved in phasic, opioid modulation of nocifensive responses, previously termed “ON” and “OFF” cells, are exclusively non-serotonergic. Therefore non-serotonergic cells that respond to noxious tail heat are referred to as ON and OFF cells below.

**Identification of serotonergic cells**

All p5HT cells were characterized using a previously described algorithm developed from an analysis of physiologically characterized, intracellularly labeled, and immunocytochemically tested cells (Mason 1997). The reliability of the classification scheme is supported by the current observation that 30 of 32 physiologically characterized, p5HT cells contained serotonin immunoreactivity. In total, of 46 p5HT cells tested since the original derivation of the classification algorithm, 43 have contained serotonin immunoreactivity (Gao and Mason 1997; Gao et al. 1997, 1998; unpublished observations). Since the possibility of our having misidentified non-serotonergic cells as serotonergic cells is small \((<10\%)\), all or nearly all of the RM and NRMC neurons studied are likely to be serotonergic. Furthermore, none of the 24 cells that have been physiologically characterized as non-p5HT and tested for serotonin content, since the original derivation of the algorithm, have contained serotonin immunoreactivity. This finding makes it highly unlikely that serotonergic RM cells have been mistakenly classified as non-p5HT. Therefore the serotonergic cells studied are likely representative of the spectrum of serotonergic cells present in RM.

**Serotonergic cells that respond to tail heat**

The present study demonstrates that 22% of serotonergic RM cells respond to noxious tail heat, with 5% of all cells being inhibited. These proportions differ from those previously reported, which themselves differ considerably. For instance, Potrebic et al. found that none of the four serotonergic cells studied were affected by noxious tail heat (Potrebic et al. 1994). However, quantitative methods were not used to analyze neuronal responses to tail heat in that study. In addition, in an effort to ensure that no response was present \((i.e.,\) that a cell was truly “NEUTRAL”), cells with small or qualitatively questionable responses were simply not studied. Therefore it is unlikely that cells with weak responses, such as those observed in the present study, were studied by Potrebic et al. In a second study using qualitative methods for analyzing tail heat responses, 5 of 13 \((38\%)\) serotonergic cells were reported to be either inhibited or excited by noxious tail heat (Mason 1997). The proportion of serotonergic cells that respond to noxious tail heat reported in the present study is likely to be more accurate than either previous estimate because of the large sample studied \((n = 150)\) and the lack of an intentional sampling bias. The current finding is also likely to be more reproducible because of the quantitative analytical methods used.

Among the serotonergic cells that changed their discharge in response to tail heat, the mean change was five to seven spikes in 10 s. In contrast, among non-serotonergic on and off cells, the mean change in discharge evoked by tail heat was 75–95 spikes. Although there is some overlap between the response magnitudes of serotonergic and non-serotonergic cells, the population values are significantly different. Furthermore, the duration of the tail heat-evoked change in discharge was shorter in responsive serotonergic than in on and off cells. These data suggest that serotonergic RM cells do not modulate nociceptive transmission following acute nociceptive stimuli. While it is clear that the responses to noxious heat observed in a minority of serotonergic cells are different from those observed in non-serotonergic on and off cells, a physiological function, if one exists, for these responses remains unclear.

For serotonergic RM cells that respond to noxious tail heat, there was no difference in the slope of the response aligned to the onset of the withdrawal or to the onset of the heat stimulus. This is of interest because Fields et al. originally argued that
off cells were likely to gate spinal nociceptive transmission in part because the tail heat-evoked inhibition of these cells was more abrupt when discharge was aligned to the onset of the tail flick withdrawal than when it was aligned to a specific tail temperature (Fields et al. 1983a). In contrast, Leung and Mason (1998) reported that the mean flick to heat slope ratio for off cells was distributed about a mean of one. Because of this finding and because of the long-lasting responses to tail heat, we proposed that the modulatory function of on and off cells is concentrated in the minutes after an acute stimulus. With regard to serotonergic RM cells, their short-duration responses and equivalent flick/heat response slopes do not support the idea that they either gate acute nociceptive information or modulate nociceptive transmission in the seconds or minutes following acute nociceptive stimuli.

As mentioned above, the response to noxious tail heat does not predict the response to opioids for serotonergic neurons as is the case for non-serotonergic cells (Barbaro et al. 1986; Fields et al. 1983b; Gao et al. 1998). For instance, non-serotonergic neurons that are inhibited by noxious heat are excited by morphine, whereas serotonergic neurons that are inhibited by noxious heat are not (Gao et al. 1998). These findings decrease the likelihood that any serotonergic cells, even those that respond to noxious tail heat, function as nociceptive-facilitatory on or nociceptive-inhibitory off cells (Gao et al. 1998). We therefore conclude that on and off cells comprise exclusively non-serotonergic cell classes. It remains possible that serotonergic cells that respond to noxious tail heat have a distinct physiological function from that of non-responsive serotonergic RM cells.

**Serotonergic cells that are unaffected by tail heat**

Most serotonergic RM and NRMC neurons do not respond to cutaneous noxious heat. This is consistent with previous reports that immunochemically confirmed and presumed serotonergic cells, including those in RM, do not respond to noxious stimuli in either anesthetized or unanesthetized animals (Auerbach et al. 1985; Jacobs and Azmitia 1992; Potrebic et al. 1994). Since there are both serotonergic and non-serotonergic cells that fail to respond to noxious tail heat, the lack of a response to tail heat cannot distinguish between serotonergic and non-serotonergic cells (Mason 1997; Potrebic et al. 1994). Instead, serotonergic cells can be identified by their slow and steady discharge pattern, a pattern that is not exhibited by non-serotonergic cells (Mason 1997). Although not explicitly stated, examination of previous publications reveals that most previously studied neutral cells have had either irregular or high discharge rates and therefore are likely to have been non-serotonergic cells (Barbaro et al. 1986; Heinricher et al. 1991, 1994; Vanegas et al. 1984). Because of serotonergic cells’ distinct physiological discharge pattern and because of their distinct neurochemistry, it is unlikely that serotonergic cells share a physiological function with non-serotonergic neutral cells. Therefore serotonergic cells, even those that do not respond to noxious stimulation, should not be considered neutral cells.

**Effect of anesthesia**

The present experiments were done exclusively in anesthetized rats and may not be indicative of serotonergic cell responses in unanesthetized animals. Chloral hydrate administration has little effect on the discharge pattern of serotonergic cells in dorsal raphe but suppresses the responses of these cells to auditory and visual stimuli (Heym et al. 1984). It is therefore possible that serotonergic RM cells that would respond to noxious heat in the unanesthetized condition are unresponsive in the lightly anesthetized rat. While this is possible, it should be noted that experiments in unanesthetized cats have also revealed that most serotonergic RM cells fail to respond to noxious stimulation (Auerbach et al. 1985).

**Functional implications**

Serotonergic RM cells comprise a distinct (by neurochemistry, morphology, and discharge properties), yet heterogeneous (by response to noxious heat), functional class. The function of serotonergic RM cells is unlikely to be similar to that of on, off, or neutral cells which should be considered exclusively non-serotonergic cell classes. Unfortunately, the neurotransmitters contained within on, off, and neutral cells remain unknown. Serotonergic RM cells’ tonic discharge pattern and blunted (or absent) response to noxious heat suggest that they are unlikely to function in the modulation of phasic nociceptive responses, such as that which occurs in the moments after a noxious stimulus (Ramirez and Vanegas 1989). Instead, serotonergic cells are likely to serve a tonic function, perhaps modulating nociceptive transmission in accordance with changing behavioral or social states (Mason 1997; Mason and Gao 1998).

The authors thank J. H. Lee and E. J. Last for technical assistance. This research was supported by National Institutes of Health Grants NS-33984 and MH-60291 and the Brain Research Foundation. Present address of K. Gao: Dept. of Psychiatry, MetroHealth Medical Center, 2500 MetroHealth Dr., Cleveland, OH 44109-1998.

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