Physiological Properties of Raphe Magnus Neurons During Sleep and Waking

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Leung, Cynthia G. and Peggy Mason. Physiological properties of raphe magnus neurons during sleep and waking. J. Neurophysiol. 81: 584–595, 1999. Neurons in the medullary raphe magnus (RM) that are important in the descending modulation of nociceptive transmission are classified by their response to noxious tail heat as ON, OFF, or NEUTRAL cells. Experiments in anesthetized animals demonstrate that RM ON cells facilitate and OFF cells inhibit nociceptive transmission. Yet little is known of the physiology of these cells in the unanesthetized animal. The first aim of the present experiments was to determine whether cells with ON- and OFF-like responses to noxious heat exist in the unanesthetized rat. Second, to determine if RM cells have state-dependent discharge, the activity of RM neurons was recorded during waking and sleeping states. Noxious heat applied during waking and slow wave sleep excited one group of cells (ON-U) in unanesthetized rats. Other cells were inhibited by noxious heat (OFF-U) applied during waking and slow wave sleep states in unanesthetized rats. NEUTRAL-U cells did not respond to noxious thermal stimulation applied during either slow wave sleep or waking. ON-U and OFF-U cells were more likely to respond to noxious heat during slow wave sleep than during waking and were least likely to respond when the animal was eating or drinking. Although RM cells rarely respond to innocuous stimulation applied during anesthesia, ON-U and OFF-U cells were excited and inhibited, respectively, by innocuous somatosensory stimulation in the unanesthetized rat. The spontaneous activity of >90% of the RM neurons recorded in the unanesthetized rat was influenced by behavioral state. OFF-U cells discharged sporadically during waking but were continuously active during slow wave sleep. By contrast, ON-U and NEUTRAL-U cells discharged in bursts during waking and either ceased to discharge entirely or discharged at a low rate during slow wave sleep. We suggest that OFF cell discharge facilitates pain-evoked reactions during sleep, whereas ON cell discharge facilitates pain-evoked responses during waking.

INTRODUCTION

Neurons in the pontomedullary raphe magnus (RM) and adjacent nucleus reticularis magnocellularis (NRMC) are important in the descending modulation of nociceptive transmission (Fields et al. 1991; Leung and Mason 1998). RM cells send axonal projections to the spinal and medullary dorsal horns where their terminals are concentrated in the superficial laminae that are known to be critical for nociceptive processing (Basbaum and Fields 1979; Basbaum et al. 1978, 1986; Holstege and Kuypers 1982). Stimulation of RM can produce either suppression or facilitation of nociceptive dorsal horn cells and nociceptive reflexes (Zhuo and Gebhart 1990, 1992), whereas lesioning or inactivation of the RM and NRMC attenuates both the suppression and the facilitation of nociceptive transmission (Behbehani and Fields 1979; Chung et al. 1987; Gebhart et al. 1983; Kaplan and Fields 1991; Sandkuhler and Gebhart 1984). These results have led to the idea that RM and NRMC contain two populations of neurons with opposing efferent function (Fields et al. 1991; Mason and Leung 1996). One population of cells is hypothesized to mediate nocicpitive inhibition such as that evoked by periaqueductal gray stimulation or opioid administration. Activation of another distinct neuronal population is thought to mediate nociceptive facilitation such as that evoked by opioid withdrawal or pyrogen administration.

As first described by Fields et al. (1983), nonserotonergic RM and NRMC cells can be divided into three classes based on their responses to a noxious thermal stimulus. OFF cells are inhibited, ON cells excited, and NEUTRAL cells unaffected by noxious thermal stimulation of the tail that evokes a withdrawal (Fields et al. 1983; Leung and Mason 1998). ON and OFF cells also have distinctive responses to a number of other physiological and pharmacological stimuli (Barbaro et al. 1986; Bederson et al. 1990; Leung and Mason 1995, 1996; Thurston and Randich 1992; Young and Dawson 1987). The consistency of cell responses among RM neurons that share the same response to noxious heat suggests that the response to noxious heat is indicative of cell function and identity. In contrast, NEUTRAL cells have heterogeneous responses to physiological and pharmacological stimuli, evidence that the NEUTRAL cell class does not represent cells of a uniform function.

The specific projection from RM and NRMC ON and OFF cells to the superficial dorsal horn suggests that these cells modulate the activity of dorsal horn cells that receive nociceptor input (Fields et al. 1995). Because OFF cell discharge consistently is correlated with antinociceptive states and ON cell discharge with enhanced nociceptive responsiveness, OFF cells are hypothesized to inhibit and ON cells to facilitate nociceptive transmission. The role of NEUTRAL cells in the modulation of nociceptive transmission has never been clear. In light of our recent demonstration that most NEUTRAL cells respond to noxious mechanical stimulation (Leung and Mason 1998), NEUTRAL cells may represent ON or OFF cell subtypes that respond only to noxious stimuli of a different intensity or modality than that commonly employed in studies of RM and NRMC cells. If this is the case, it would necessarily imply that the response to opioids is not entirely homogeneous among ON and OFF cells.

The above electrophysiological description of ON, OFF, and
**NEURAL** cells is based on the results of experiments performed in anesthetized animals. Studies examining the effects of noxious somatic stimuli on RM cells of unanesthetized rats have identified on-like cells and neutral-like cells, but no off-like cells (Martin et al. 1992; Oliveras et al. 1990). In these studies, cells that are inhibited by noxious stimulation only were observed after induction of barbiturate anesthesia (Oliveras et al. 1991a,b). These results have been interpreted as evidence that on cells are inactive in the unanesthetized condition and are revealed by anesthesia. This data presents a serious challenge to the idea that two RM cell populations, defined by their responses to noxious stimulation, modulate nociception in opposing directions. To determine whether on-like cells exist in the unanesthetized animal, we tested the response of RM neurons to noxious stimulation during waking and sleeping states.

RM and NRMC neurons have been implicated in the modulation of numerous physiological processes besides spinal nociceptive sensitivity (Mason and Leung 1996). It is interesting to note that all of the homeostatic, sensory, and motor processes that have been proposed as targets of RM/NRMC cell modulation vary across the sleep/wake cycle (Kleitman 1963). Nonserotonergic as well as serotonergic RM/NRMC cells have state-related discharge in the cat (Cespuglio et al. 1981; Kanamori et al. 1980; Sakai et al. 1983; Sheu et al. 1974; Steriade et al. 1984). To determine whether RM cells that respond to noxious stimulation have state-related discharge, the activity of RM cells was recorded during slow wave sleep (SWS) and waking in the rat.

**METHODS**

**Surgical preparation for chronic recordings**

Male Sprague-Dawley rats (400–500 g; Sasco, Madison, WI) were instrumented for chronic recording. Rats were anesthetized with nembutal (55 mg/kg) and placed in a stereotaxic apparatus. The methods for recording electroencephalographic (EEG) and electromyographic (EMG) measures were adapted from Bergmann et al. (1989). Briefly, two pairs of bone screws were inserted through the frontal and parietal bones for differential electroencephalographic recording. Cortical activity (EEG) was optimized in recordings from two laterally placed bone screws and hippocampal theta activity was optimized in recordings from two medially placed bone screws (Bergmann et al. 1989). Stranded stainless steel wires were inserted through the biceps femoris and the deep muscles of the neck for EMG recording (Bergmann et al. 1989). A threaded microdrive base and guide tube (19 gauge) were affixed to the skull overlying the RM (centered at P 1.0–2.6 mm from interaural zero, L 0–1.0 mm) for extracellular unit recording. The threaded base (for a microdrive) was fixed in place and thus allowed for a single electrode trajectory in each animal. EEG, theta, and EMG leads were attached to a miniature connector, which was affixed to the skull with dental acrylic. Rats were allowed to recover for ≥1 wk.

**Experimental protocol during anesthesia**

In a typical recording session, rats were anesthetized for a total of 0–2 h. This 2 h included: 30 min for anesthetic concentration to reach a steady state, during which no recordings were made; ≥45 min during which a single-unit recording was isolated; and 45 min to characterize the cell’s background discharge and responses to noxious heat. Anesthesia then was discontinued. After discontinuation of anesthetic administration, a minimum of 1 h, and a maximum of 2 h, was needed for rats to show normal waking behavior and physiology.

The length of this transition time was related directly to the total time that a rat spent anesthetized. Recordings from the transition state were used only for ensuring constancy of the extracellular waveform and were not otherwise analyzed. When a unit was lost, the microdrive was advanced and another cell isolated. Animals always entered waking from the transitional state. All recordings then were maintained for as long as possible (1–6 h) in the unanesthetized condition. Rats naturally cycled through waking, SWS, and occasionally paradoxical sleep (PS; also known as rapid-eye movement or desynchronized sleep) states. In preliminary isolated recordings, we observed that rats will not sleep if noxious heat is applied more than about five times in an hour. Therefore in an attempt to obtain recordings during sleep states, noxious heat was applied infrequently (<5/h) and irregularly. Details of this protocol follow.

Rats were anesthetized with isoflurane delivered via nose cone. A microdrive-microelectrode assembly (Biela Idea Development, Anaheim, CA) was attached to the threaded base (Deadwyler et al. 1979; Malpeli et al. 1992). EMG electrodes were placed transcutaneously in the paraspinal muscles. The miniature connector was attached to recording equipment via a flexible cable mounted on a counterbalanced boom.

The concentration of isoflurane was allowed to equilibrate at 1.10 –1.25% for ≥30 min. A metal microelectrode (FHC, New Brunswick, ME) then was advanced into the region of the RM (V 8.5–10.5 mm). No search stimulus was used, and only units with background discharge were studied. Every extracellular unit that was isolated and had a signal-to-noise ratio of ≥4 was studied. Background discharge for each neuron was recorded for 15 min in the absence of stimulation and at a steady-state concentration of isoflurane.

The neuronal response to four to nine applications of noxious heat was recorded. We used two alternate methods for noxious heat stimulation. In early experiments, radiant heat was applied using a modified Hargreaves apparatus (Hargreaves et al. 1988). This apparatus consisted of a focused light bulb that produced a ramping thermal stimulus that evoked paw and tail withdrawals at latencies of 3–5 s and 1–3 s, respectively. This technique had the disadvantage that the stimulus duration, and thus magnitude, was different in each trial. In later experiments, a CO₂ laser was used to apply fixed pulses of noxious heat. The advantage of this method is that the laser pulse is shorter than the minimal time required for the rat to withdraw. The fabricated CO₂ laser stimulator is similar to the instrument originally described by Mor and Carmon (1975). Importantly, this device was constructed so that the beam from the laser could be aligned to any location within the plane of the cage floor. The laser stimulus heated a very small (~1 mm diameter) spot of skin at an average rate of >75°C/s (Haimi et al. 1983). Thus a 300- to 320-ms pulse from the CO₂ laser (2.5 W) was used in heating either the hindpaw or the tail. Such a pulse evoked a brisk withdrawal followed by licking when applied to awake rats and a brief burning pain when applied to the experimenters’ fingertip pads.

The heat stimulus was applied at 3-min intervals. The only exception to this schedule was in the case of cells that appeared to be inhibited by prior heat stimulation (candidate on cells) and had no background discharge at the time of the trial. In such cases, the trial was performed after one or more additional 3-min intervals, when discharge resumed. After characterization of the neuronal responses to noxious heat, cells were characterized further by their responses to innocuous brush stimulation. A paint brush was stroked gently along the rat’s back, hindlimbs and tail. In some cases, cell discharge also was recorded in response to a hand clap.

**Spike acquisition and discrimination**

As mentioned in the previous section, metal microelectrodes (FHC, New Brunswick, ME) were used to record all extracellular units that initially had a signal-to-noise ratio of ≥4. A preamplifier was contained within the cable and was used to amplify the unit recording.
10-fold (Szabo and Marczynski 1993). The unit record then was amplified 1000-fold by an AC amplifier (Warner Instruments, Hamden, PA) and digitized at 20 kHz by a CED 1401+ interface (Cambridge Electronic Design, Cambridge, UK). Spike2 acquisition software (Cambridge Electronic Design) stored the time of the spike, the spike type, and 30 digitized points from the waveform. Individual waveforms were reviewed off-line when each spike’s assignment to a particular unit was checked using template-matching software (see Fig. 9). Although it was possible to record from spikes that matched more than one template (i.e., to record multiple single units), multiple recordings rarely occurred.

Transition from anesthesia to waking

Because low levels of anesthesia produce a marked behavioral excitability, rats that were allowed to recover from anesthesia were hyperexcitable during the transition to waking. This behavior was manifest by rapid walking and turning within the recording chamber, often accompanied by jerking motions of the head. Consequently, in preliminary experiments, many neurons were lost during the transition. To blunt this hyperexcitability and increase the number of cells held through to the awake state, a low dose of the quickly metabolized, benzodiazepine, midazolam (1–2 mg/kg sc), was administered 90 s before termination of the anesthetic (Hovinga et al. 1992; Mandema et al. 1992). Recovery from the midazolam-induced transition state was assessed by behavioral, EEG, and EMG criteria and typically required 1–2 h. During the transition, animals were behaviorally quiescent as during SWS. However, the transition state was differentiated from natural SWS by an atypical posture (i.e., limbs splayed out with eyes open), a greater amplitude EEG and lower amplitude nuchal EMG activity.

Spike shape and amplitude were monitored continuously throughout the transition from anesthesia to waking. Although spikes almost always changed in amplitude, recordings were continued as long as the waveform could be continuously identified and distinguished from newly appearing waveforms. When the animals had recovered fully from anesthesia, neurons were tested again, at irregular intervals, for their responses to noxious heating of the hindpaws and tail using either radiant or laser heat stimulation.

Rats spontaneously cycled through waking, SWS, and PS states. Behavioral states were determined on the basis of established EEG and EMG criteria (Bergmann et al. 1987, 1989). Waking was indicated by low-amplitude EEG, high-amplitude nuchal EMG, and low-amplitude theta rhythm. SWS was marked by high-amplitude EEG, low-amplitude nuchal EMG, and high-amplitude theta rhythm. PS was characterized by low-amplitude EEG, low-amplitude nuchal EMG, and high-amplitude theta rhythm. Occasionally, rats had a low-amplitude EEG, low-amplitude nuchal EMG, and low-amplitude theta rhythm, indicating a drowsy-like state.

To maximize the probability that animals would enter into SWS and PS states, animals were habituated to the recording apparatus by spending several hours in the recording chamber for ≥3 days before recording. Furthermore, as mentioned above, noxious and innocuous stimuli were applied infrequently and irregularly.

Cell characterization

The metal electrodes used in this study appeared to be heavily biased toward nonserotonergic cells. Of the 60 cells characterized in the anesthetized rat for this experiment, none discharged in the slow and steady manner that is characteristic of serotonergic cells (Mason 1997). Nonetheless, we checked that all cells were nonserotonergic by analyzing their background discharge using a previously defined algorithm with <10% error rate (Mason 1997).

The response to noxious heat was analyzed as previously described (Leung and Mason 1998). Briefly, the magnitude of unit responses to heat was calculated as the difference between the unit firing rate before and after stimulus application. The magnitude of the change in discharge evoked by tail heat was compared with the variability of the background discharge. The variability of the background discharge was quantified as the standard deviation of the change between sequential bins of background discharge normalized to impulses per second (SDbin). Heat-evoked increases in discharge that were >2 SDbin were considered excitatory responses, and evoked decreases in discharge that were >2 SDbin were considered inhibitory responses. The response period was 4 s for laser heat stimulation and 10 s for radiant heat stimulation.1 Thus for laser stimuli, responses were calculated as the difference in unit firing rate between a 4-s response period and a 4-s period immediately before the stimulus was applied. This evoked response was compared with the SDbin calculated from 4-s bins.

Because the discharge of RM cells was observed to be state dependent (see Spontaneous discharge patterns in the unanesthetized conditions), the variability of the spontaneous discharge was calculated for each state—anesthesia, waking, and SWS. There were insufficient recordings during PS for statistical analysis. Evoked changes in discharge then were compared with response thresholds that were determined from the spontaneous discharge of the appropriate behavioral state.

Cells that were excited by ≥50% of at least three heat applications during either SWS or waking were considered ON-U cells. Similarly, cells that were inhibited by ≥50% of at least three heat trials during either SWS or waking were considered OFF-U cells. Cells with <50% excitatory or inhibitory responses to noxious stimulation during waking and SWS were classified as NEUTRAL-U cells. Cells that were not excited or inhibited by ≥50% of the trials applied during waking and were not tested with at least three heat trials during SWS were unclassified.

Statistics

Each variable is expressed as a mean ± SE. Statistical tests were performed using SAS (Cary, NC), Microsoft Excel (Redmond, WA) or Statview (Berkeley, CA).

Histology

The recording depth for every unit was recorded. 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 distance from the marked recording site. Animals then were overdosed and perfused with fixative. 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

All cells (n = 37) recorded in unanesthetized rats were contained in RM or the adjacent NRMC (Newman 1985a,b) between the facial nucleus and the caudal pole of the trapezoid body (Fig. 1). Cells were classified as ON-U (n = 14), OFF-U (n = 7), or NEUTRAL-U (n = 7) cells (see METHODS). Nine cells could not be classified. ON-U, OFF-U, and NEUTRAL-U cells were not preferentially distributed within the RM and NRMC region.1

1 Because the magnitude of the neuronal response to the radiant heat stimulus was not correlated to the length of the stimulus application (not shown), the variance in stimulus duration did not have a consistent biasing effect on the neuronal response.
Noxious heat-evoked responses

ON-U (n = 14) and OFF-U cells (n = 7) were observed in both waking and SWS states in unanesthetized rats. As shown in Fig. 2, ON-U cells increased their discharge by 6–48 impulses/s in response to noxious stimulation. The response magnitude varied during waking (Fig. 2, A and B), and the mean ON-U cell response magnitude was not significantly different between waking and SWS. OFF-U cells decreased their discharge by 4–59 impulses/s in response to noxious stimulation applied during waking or SWS (Fig. 3). The mean OFF-U cell response magnitude was not significantly different between waking and SWS states. For both ON-U and OFF-U cells, responses to noxious stimulation were consistent across all sites tested (tail, bilateral hindpaws).

NEUTRAL-U cells (n = 7) did not respond to noxious stimulation during either waking or SWS. An additional nine cells did not respond to noxious stimulation during waking but were not tested with a sufficient number of trials during SWS to be classified (see METHODS).

The proportion of noxious heat trials that elicited OFF-U and ON-U cell responses differed according to the behavioral state of the rat at the time of stimulus onset (Fig. 4). OFF-U cells (n = 6) responded to 97 ± 3% of the heat trials applied during SWS and only 44 ± 13% of those applied during waking (Fig. 4B). Similarly, ON-U cells (n = 13) responded to a larger percentage of noxious heat trials applied during SWS (92 ± 4% of the trials) than during waking (37 ± 9% of the trials; Fig. 4A). Both ON-U and OFF-U cells were more responsive to noxious heat applied during SWS than during waking (χ² tests, P < 0.001). Noxious heat stimulation evoked motor withdrawals when applied during either waking or SWS (Figs. 2 and 3).

Context dependence of noxious-evoked responses

Within the waking state, responses to noxious stimulation were variable and sometimes appeared to be influenced by activities that “distracted” the rat from the stimulus. ON-U (n = 3) and OFF-U (n = 3) cells were significantly less likely to respond to noxious heat trials initiated during eating and drinking (17 ± 11%) than to trials initiated under other waking conditions (46 ± 10%; χ², P < 0.001). The motor response to noxious stimulation also was smaller during distracting activities compared with other waking conditions in five of seven rats. For example, Fig. 5 shows that when laser stimulation was applied while the rat was drinking, the OFF-U cell inhibition was blocked and motor response reduced compared with the response evoked when the rat was standing quietly.

Spontaneous discharge patterns in the unanesthetized condition

Of the 37 cells recorded in the unanesthetized condition, 32 cells were recorded during both SWS and waking states. Ninety percent of these neurons (29/32) exhibited behavioral state-related changes in spontaneous discharge. Neurons with state-dependent changes in discharge (n = 29) were catego-
rized as having one of two distinct spontaneous activity patterns: wake-active or SWS-active. The majority of neurons ($n = 21$) were encompassed by the wake-active pattern illustrated in Fig. 6. Neurons in this group exhibited higher spontaneous discharge rates during waking ($12.4 \pm 2.7$ Hz) than during SWS ($2.8 \pm 0.8$ Hz). For wake-active neurons, the waking discharge was sporadic in nature with the largest bursts of activity occurring during isolated movements of the head, neck, jaw, or limb and in conjunction with gross movements of the entire body (e.g., walking, standing). Neurons either ceased to discharge entirely or discharged at a low level during SWS. The activity of three wake-active neurons was recorded during PS. In every case, the neuronal activity during PS was equal to or greater than that observed during waking.

**FIG. 2.** An ON-U cell’s responses to laser heat stimulation of the hindpaw during waking (A and B) and slow wave sleep (SWS; C). In each panel, the traces, from top to bottom, are neuronal response (instantaneous frequency), limb electromyographic (EMG) trace, stimulus application, neck EMG and electroencephalogram (EEG) as labeled in A. A and B: 2 different trials of noxious heat show the variable ON-U cell response. C: response to a laser heat trial applied during SWS. Time calibration, shown in C, is applicable to all panels.
The remainder of the neurons ($n = 8$) showed a SWS-active discharge pattern (Fig. 7). SWS-active neurons exhibited sporadic waking discharge but typically were inhibited during spontaneous movements (see Fig. 8B). In contrast to wake-active neurons, SWS-active neurons became continuously active during SWS. The average discharge during SWS (33.2 ±

**FIG. 3.** OFF-U cell’s responses to laser heat stimulation of the hindpaw during waking (A) and SWS (B). In each panel, the traces, from top to bottom, are neuronal response (instantaneous frequency), limb EMG trace, stimulus application, neck EMG, and EEG as labeled in A. A: response to a trial of noxious heat during waking. B: response to a laser heat trial applied during SWS. Time calibration, shown in B, is applicable to both panels.

**FIG. 4.** For both ON-U (A) and OFF-U (B) cells, the probability of a response to noxious heat stimulation is greater during SWS (sws) than during waking (w).
8.1 Hz) was greater than that during waking (7.9 ± 1.7 Hz) although the peak discharge rates during waking were often greater than those during SWS (Fig. 7). The activity of two SWS-active neurons was recorded during PS. In both cases, the spontaneous discharge rate was lower during PS than during either waking or SWS. For both SWS- and wake-active cells, changes in neuronal activity were nearly coincident with changes in EEG associated with SWS-to-wake or wake-to-SWS transitions (Figs. 6 and 7).

Wake-active and SWS-active neurons were segregated by cell class ($\chi^2$, $P < 0.001$). Most ON-U (11/13), NEUTRAL-U (3/5), and unclassified (7/8) cells exhibited the wake-active pattern (Table 1). All OFF-U cells (6/6) exhibited the SWS-active discharge pattern.

Because ON-U cells discharged preferentially during waking, whereas OFF-U cells were relatively inactive during waking, it is possible that the ON-U and OFF-U responses to noxious stimulation during SWS were not stimulus specific but rather were secondary to a heat-evoked state change. However, examination of cortical activity during noxious stimulation trials revealed that cortical desynchronization typically did not occur until hundreds of milliseconds to seconds after the initiation of the motor and neuronal responses (Figs. 2C and 3B). In addition, one ON-U cell that was atypical in that it showed a SWS-active discharge pattern exhibited a biphasic response to noxious stimulation (not shown). This cell’s response to a noxious stimulus applied during SWS consisted of an initial excitation concurrent with the withdrawal reflex, followed by an inhibition at the time of cortical desynchronization.

FIG. 5. Motor and neuronal responses to noxious stimulation applied during waking were suppressed during drinking. A: OFF-U cell was inhibited by laser stimulation of the hindpaw when awake and standing quietly. B: laser stimulation applied while the rat was drinking did not elicit a response in this OFF-U cell. Time calibration, shown in A, is applicable to both panels.

FIG. 6. ON-U cell discharge during SWS and waking. Traces, from top to bottom, are neuronal activity (instantaneous discharge), a line indicating SWS periods, EEG, nuchal EMG, and hippocampal theta. Spontaneous discharge in the absence of anesthesia. This ON cell was active in bursts during waking and relatively inactive during SWS.
FIG. 7. OFF-U cell discharge during SWS and waking. Traces, from top to bottom, are neuronal activity (instantaneous discharge), a line indicating SWS periods, EEG, nuchal EMG, and hippocampal theta. Spontaneous discharge in the absence of anesthesia. This OFF cell discharged steadily and continuously during SWS and despite occasional bursts was less active during waking.

FIG. 8. In the unanesthetized condition, ON-U (A) and OFF-U (B) cells responded to innocuous somatosensory and auditory stimulation. A: ON-U cell was excited by brush of the hindpaw (left) and by clap (right). 2, approximate timing of each brush stroke and of the clap. B: OFF-U cell was inhibited by brush of the hindpaw (left) and during spontaneous movements (right). The time calibration, shown in the right part of A, is applicable to all panels.
As mentioned in the preceding text, ON-U cells were excited and excitatory response to innocuous somatic stimulation (Fig. 8 clap) increased the discharge of most cells (4/6) that had an in the anesthetized condition. Auditory stimulation (a loud stimulus in the absence of anesthesia. The distribution of

Notably, all five of the cells that were inhibited by radiant or characteristic response to noxious thermal stimulation (Table 2). For cells belonging to the ON, OFF, or NEUTRAL cell classes (Table 2). The proportion of neurons held after recovery was similar after recovery from anesthesia and the transitional state (Fig. 9). The proportion of neurons held after recovery was similar for cells belonging to the ON, OFF, or NEUTRAL cell classes ($\chi^2$, $P > 0.3$).

Of the 17 cells that were characterized under both anesthetized and unanesthetized conditions, 12 retained their characteristic response to noxious thermal stimulation (Table 2). Notably, all five of the cells that were inhibited by radiant or laser heat in the anesthetized state were inhibited by the same stimulus in the absence of anesthesia. The distribution of responses was significantly different from what would be expected by chance ($\chi^2$, $P < 0.002$).

**Responses to innocuous stimulation**

Most neurons were completely unresponsive to brush stimulation in the anesthetized condition (Leung and Mason 1998). In the few neurons that did respond to innocuous stimulation, the evoked responses were smaller in magnitude than responses evoked by noxious stimulation. In contrast, most cells tested (11/13) during waking or SWS were responsive to innocuous tactile stimulation. Innocuous stimulation elicited excitatory responses from five ON-U and two unclassified cells and inhibitory responses from one OFF-U and one unclassified cell. Responses to innocuous stimulation in the unanesthetized rat were similar in magnitude to noxious heat-evoked responses. ON-U cells burst in response to each brush stroke (arrows in Fig. 8A), whereas OFF-U cells were inhibited by brush (Fig. 8B). It should be noted that the ON-U cell shown in Fig. 8A was completely unresponsive to brush of the hindpaws in the anesthetized condition. Auditory stimulation (a loud clap) increased the discharge of most cells (4/6) that had an excitatory response to innocuous somatic stimulation (Fig. 8A).

As mentioned in the preceding text, ON-U cells were excited and OFF-U cells inhibited during self-generated movements, possibly due to proprioceptive input (Fig. 8B).

**Comparison of noxious responses during anesthetized and unanesthetized states**

Only 20 of the 60 cells recorded during anesthesia were held after recovery from anesthesia and the transitional state (Fig. 9). The proportion of neurons held after recovery was similar for cells belonging to the ON, OFF, or NEUTRAL cell classes ($\chi^2$, $P > 0.3$).

Of the 17 cells that were characterized under both anesthetized and unanesthetized conditions, 12 retained their characteristic response to noxious thermal stimulation (Table 2). Notably, all five of the cells that were inhibited by radiant or laser heat in the anesthetized state were inhibited by the same stimulus in the absence of anesthesia. The distribution of responses was significantly different from what would be expected by chance ($\chi^2$, $P < 0.002$).

**Discussion**

In the present study, a small number of spontaneously active neurons were studied. Furthermore, because we depended on rats’ naturally cycling through sleeping and waking states and because recordings are difficult to maintain in unrestrained rats, the protocol was not completed for all cells. Despite these limitations, our findings provide answers for the two questions set out in the introduction. First, OFF-like cells that are inhibited by noxious stimulation exist in the unanesthetized animal. Second, cells with a common response to noxious heat also share a state-related spontaneous discharge pattern. Specifically, ON-U cells that are excited by noxious stimulation are more active during waking than during SWS, whereas OFF-U cells that are inhibited by noxious heat are more active during SWS than waking. The state-related spontaneous discharge patterns of ON-U and OFF-U cells is evidence that these cells play an important role in state-dependent modulation of nociceptive processes.

In addition to the conclusions above, several intriguing results merit further study. Future experiments are necessary to better elaborate the state- and context-dependent responses of RM cells during PS may require sleep deprivation to increase the likelihood of rats entering the PS state. Finally, recording the responses of NEUTRAL-U cells to a more varied set of noxious stimuli would aid in determining whether NEUTRAL-U cells are truly neutral.

**Comparison with previous results**

**Heat-evoked responses in the unanesthetized rat.** One other group of investigators has recorded the noxious stimulus-evoked responses of nonserotonergic RM/NRMC neurons in the awake, freely moving rat. These investigators found only neutral-like and ON-like cells (i.e., cells excited by noxious stimulation and response to noxious stimulation diminished by morphine) (Martin et al. 1992; Oliveras et al. 1990). The ON-like cells recorded by Oliveras were excited by both noxious and innocuous somatic stimulation applied to widespread areas of the periphery and also were activated by auditory stimulation (Oliveras et al. 1989, 1990, 1991a,b). Similarly, the ON-U cells recorded in the present experiment were excited by brush, noxious heat, and clap and did not show a restricted cutaneous receptive field. In further agreement with Oliveras,
we found that the ON-U cell was the most prevalent RM cell type in the unanesthetized rat.

Although it is possible that the NEUTRAL-U cells recorded in the present study and the NEUTRAL-like cells recorded by Oliveras are unresponsive to any form of noxious stimulation and are not involved in nociceptive modulation, it is more likely that these cells are in fact responsive to other types of noxious or somatic stimulation. In support of this idea, 67% of the cells characterized during anesthesia as NEUTRAL, by their lack of response to noxious heat, respond to noxious mechanical stimulation (Leung and Mason 1998). In the unanesthetized rat, NEUTRAL-U cells share other physiological properties with ON-U cells, such as the wake-active discharge. These results suggest that the NEUTRAL cell category may be an artifact of the limited noxious stimulation paradigms employed and that NEUTRAL cells comprise a subclass of ON and OFF cells that respond to only a subset of cutaneous noxious stimuli.

Oliveras’ group did not observe cells with OFF-like properties in the unanesthetized state. Because OFF-like cells were observed only after induction of barbiturate anesthesia, it was proposed that OFF cells are not active in the unanesthetized condition and are revealed by anesthesia (Oliveras et al. 1991a,b). In contrast to previous findings (Oliveras et al. 1991a,b), the results of the present study indicate that cells that are inhibited by noxious stimulation are not unique to the anesthetized state. Because OFF-U cells were inhibited by both noxious and innocuous stimuli, the failure to find OFF-like cells in previous studies is likely due to the experimental protocols used by Oliveras’ group. For instance, Oliveras et al. (1989) presented 10 repetitions of each stimulus type with an intertrial interval of 15 s for auditory and mechanical (both noxious and innocuous) stimuli and an interval of 3 min for noxious thermal stimuli. This protocol of repeated and frequent peripheral stimulation, which would inhibit OFF-U cells, at least in part may account for previous failures to record OFF-like cells in the unanesthetized rat. In support of the above reasoning, spontaneously active RM units that are excited by morphine (an OFF cell characteristic) have been recorded in awake, unstimulated animals (McGarauthy et al. 1993). Furthermore, Oliveras’ group only studied rats in the waking state. As shown in the present study, OFF-U cells have only sporadic discharge during waking, and even when active, they are less likely to respond to noxious stimulation during waking than during SWS.

STATE-DEPENDENT DISCHARGE. Several previous studies have examined the state-dependent discharge of nonserotonergic RM and NRMC cells in the cat (Cespuglio et al. 1981; Kanamori et al. 1980; Sakai et al. 1983; Sheu et al. 1974; Steriade et al. 1984). Most RM and NRMC cells were more active during waking than during SWS and were most active during PS. This wake-active pattern of spontaneous activity is consistent with the predominant pattern observed in two previous studies in the cat (Sheu et al. 1974; Steriade et al. 1984). However, other studies in the cat have reported RM and NRMC cells that discharge more rapidly during PS than during either SWS or waking (Cespuglio et al. 1981; Kanamori et al. 1980; Sakai et al. 1983). In the present study, few cells discharged equally during SWS and waking.

The correlation between noxious-stimulus evoked responses and state-related spontaneous discharge patterns reported in the present study is novel. It is possible that the state-related discharge of RM ON-U and OFF-U cells is secondary to an autonomic change. In the anesthetized rat, ON-U and OFF-U cells burst in reciprocal relation to changes in blood pressure (Leung and Mason 1996). Alternatively, it is possible that RM and NRMC cell state-related discharge is a response to peripheral afferent input. Thus during waking, somatic input would lead to the activation of ON-U cells and the inhibition of OFF-U cells. During SWS, in the absence of self-generated movements, somatic input would decline, resulting in inactive ON-U cells and disinhibited OFF-U cells. In support of this idea, most RM cells discharge at similar rates during SWS and waking in spinalized cats (Cespuglio et al. 1981).
simultaneous fluctuations, in the predicted direction, of the tail flick latency (Heinricher et al. 1989). Thus the tail flick latency was longer when heat was applied during OFF cell discharge than when applied during ON cell discharge. These findings then predict that withdrawals should be suppressed during SWS, when OFF-U cells are active. However, in a previous study from this lab, the converse was observed (Mason and Escobedo 1995). Noxious heat-evoked paw withdrawals occur at a shorter latency during SWS than during waking. Furthermore in the present study, withdrawal movements were elicited by noxious stimulation applied during SWS as well as waking.

A parsimonious resolution to this apparent paradox is that ON and OFF cells modulate a reaction to noxious stimulation other than the flexion withdrawal. An intriguing possibility is that ON and OFF cells preferentially affect the alerting reaction evoked by noxious stimulation. Although the specific anatomic locus for noxious stimulus-evoked alerting is unknown, it must lie supraspinally, whereas the principal circuitry for the motor withdrawal is spinaly located. A recent anatomic study suggests that the superficial dorsal horn neurons transneuronally labeled from tail withdrawal muscles are rarely retrogradely labeled from thalamus (Jasmin et al. 1997). Therefore the neurons mediating motor responses to noxious stimulation may be distinct from those that project to forebrain and are involved in arousal and alerting behaviors. In support of such a distinction, stimulation of flexor reflex muscle afferents elicits a flexion reflex during SWS at a lower intensity than that required for cortical arousal (Giaquinto et al. 1964).

The alerting response evoked by noxious stimulation may be modulated by behavioral state. In the present study, when noxious heat was applied during SWS, rats typically raised their head briefly and then returned to SWS within seconds. In contrast, noxious heat applied during waking evoked longer-lasting changes in behavior, often prompting rats to look around the recording chamber, move to a new location and change body position. Similarly, in humans, cutaneous pain rated at 5.1 on a 10-point visual analogue scale and described as “burning” and “miserable” when applied during waking failed to evoke EEG changes when applied during SWS (Drewes et al. 1997). Such a suppression of noxious stimulus-evoked arousals during SWS may be an important homeostatic mechanism for ensuring that sufficient time is spent in the SWS state.

Functional implications of state-dependent heat-evoked activity

Both ON-U and OFF-U cells were more likely to respond to noxious stimulation applied during SWS than during waking. During SWS, the noxious stimulus-evoked excitation of ON-U cells and inhibition of OFF-U cells would facilitate the response to another stimulus applied shortly afterward. This idea leads to the prediction that repeating a nonarousing noxious stimulus at a short interval, when OFF-U cells are likely still inactive and ON-U cells still bursting, should evoke arousal. Such an effect has been reported in both animals and humans (Drewes et al. 1997; Giaquinto et al. 1964) and may serve as an important protective mechanism.

The authors thank A. Zhang, D. Chen, and C. Burgin for technical assistance, Dr. Dottie Hanck for help with SAS and Drs. Donna Hammond, Bob McCrea, and Jay Goldberg for helpful conversations over the course of the study.

This research was supported by the Brain Research Foundation and National Institute on Drug Abuse Grant DA-07861. C. G. Leung was supported by Grant DA-05698 and by a grant from the Women’s Council of the Brain Research Foundation. Isoflurane was generously provided by Anaquest Corp.

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Received 23 July 1998; accepted in final form 6 October 1998.

REFERENCES


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