Nociceptive Responsiveness During Slow-Wave Sleep and Waking in the Rat

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Abstract: Brainstem neurons that are thought to modulate pain are reported to have state-dependent discharge rates. Yet, the effect of behavioral state upon nociceptive transmission has not been well studied. Therefore, we examined responses to noxious thermal stimulation of the rat hindpaw presented during different behavioral states. Noxious thermal stimuli were applied to rats as they spontaneously cycled through waking and sleeping states. Two different methods of heating the paw—a focused light bulb (“radiant heat”) and a CO2 laser (“laser heat”)—were employed. Regardless of the heating method used, rats withdrew from noxious thermal stimulation when it was applied in each behavioral state tested. When rats were tested with radiant heat, the withdrawal latency from noxious heat was shorter during slow-wave sleep than during waking. In contrast, when tested with laser heat, there was no difference in either the response latency or magnitude evoked by noxious heat across sleep/wake states. Despite the fact that rats withdrew from noxious heat (using either method of application) applied during sleep, the rats quickly returned to sleep afterwards. The latency to sleep after noxious stimulation was significantly greater during waking than during sleeping.

The behavioral response to noxious thermal stimulation includes both an initial motor withdrawal which is enhanced during sleep and arousal or alerting which is suppressed during sleep. Therefore, pain evokes at least two distinct reactions that are differentially modulated across sleep/wake cycles.

Key words: Pain modulation; nociception; antinociception; monoamines; serotonin; norepinephrine; raphe magnus

INTRODUCTION

PAINFUL STIMULI EVOKE MOTOR, AUTONOMIC, AFFECTIVE, AND COGNITIVE REACTIONS THAT SERVE TO PROTECT AN ORGANISM AND PROLONG ITS LIFE. The most advantageous reaction to pain is dependent on a myriad of factors including the intensity, location, and modality of the painful input as well as posture, autonomic tone and the behavioral context of the animal at the time of injury. The majority of studies on pain have been performed in anesthetized animals and only a few studies have examined how behavioral context affects the reactions to painful stimuli.1,2 The present study examines the responses to pain during sleep and waking.

Brainstem cells that modulate pain include serotonergic and non-serotonergic neurons in raphe magnus and the adjacent ventromedial reticular formation (collectively abbreviated as RM), noradrenergic neurons in the locus coeruleus (LC), A5 and A7 groups, and cells of heterogeneous neurochemistry in the midbrain periaqueductal gray.3,7 Many of these pain modulatory cells, both monoaminergic and non-monoaminergic, discharge in a state-dependent manner.8-10 Serotonergic RM and noradrenergic neurons are most active during alert waking and least active during paradoxical sleep with intermediate discharge rates observed during quiet waking and slow wave sleep. The preponderance of data suggests that the overall effect of increased serotonin or norepinephrine release within the dorsal horn leads to a decrease in nociceptive transmission.3,6,7 One would then predict that antinociception would be more pronounced during the waking state, when monoaminergic tone is at its highest, than during sleep states when monoaminergic tone is lower. In order to test that prediction, we tested the latency to noxious heat-evoked paw withdrawal during slow-wave sleep and waking in rats.

RM contains two non-serotonergic populations that have been implicated in pain modulation.3,4 Specifically, OFF cells are excited by opioids and are thought to suppress nociceptive transmission while ON cells are inhibited by opioids and may facilitate nociceptive transmission. We have recently demonstrated that ON and OFF cells have state-dependent discharge in unanesthetized, behaving rats.10 OFF cells are continuously active during slow-wave sleep, only sporadically active during waking, and virtually silent during paradoxical sleep, whereas ON cells are active during waking, virtually silent during slow-wave sleep, and most active during paradoxical sleep. These discharge patterns combined with the hypothesized function of ON and OFF cells predict that antinociception would accompany slow-wave sleep, when OFF cells are most active. Clearly, this prediction is contrary to the prediction based on monoaminergic cell discharge (see above). Therefore, in the present study, we recorded the behavioral reactions of rats to noxious thermal stimuli presented during slow-wave sleep or waking states.

MATERIALS AND METHODS

Male Sprague Dawley rats (Sasco, Madison, WI) were used in...
all experiments. Rats tested with radiant heat were prepared for chronic electroencephalographic (EEG) and electromyographic (EMG) recordings (n=7). Rats tested with laser heat were prepared for chronic EEG and EMG recordings (n=8) or only for EMG recordings (n=9). In animals instrumented for both EEG and EMG recordings, both measures were used in determining behavioral state (see criteria below). In the remaining animals, video tapes synchronized to the EMG recording were used to determine behavioral state (see criteria below).

Surgical Preparation. Rats were anesthetized with nembutal (55 mg/kg) and placed in a stereotaxic apparatus. The methods for recording EEG and EMG measures were adapted from Bergmann et al. Briefly, two pairs of bone screws were inserted through the frontal and parietal bones for differential recording. Cortical activity was optimized in recordings from two laterally placed bone screws and hippocampal theta activity was optimized in recordings from two medially placed bone screws. Stranded stainless steel wires were inserted through the biceps femoris bilaterally, the paraspinal muscles, and/or the deep muscles of the neck for EMG recording. 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 at least one week.

Experimental Testing. Rats were habituated to the testing chamber for at least five days. Rats were always tested between 09:00 and 17:00 during the light portion of their daily cycle (05:30 on, 18:00 off) when rats typically sleep. Ambient temperature was maintained at 23-23.5°C except where explicitly stated.

We used two alternate methods for noxious heat stimulation. Noxious radiant heat was applied using a modified Hargreaves apparatus. This apparatus consisted of a focused light bulb with a beam that passed through a thin (2 mm) sheet of glass. The device produced a ramping thermal stimulus which evoked paw withdrawals at latencies of 5—10 seconds. This technique had the disadvantage that the stimulus duration, and thus magnitude, was different in each trial. In later experiments, a CO₂ laser stimulator, similar to that described by Mor and Carmon, 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 laser stimulus heated a very small (~1 mm diameter) spot of skin at an average rate of 75-300 °C/s depending on the wattage used. Thus, in order to produce a brief, burning pain when applied to the experimenters’ fingertips, a 300—320 ms pulse at 2.5 W or a 80 ms pulse at 10 W was needed. Pulses that evoked pain in the experimenters also evoked a brisk withdrawal followed by licking when applied to awake rats.

Rats were tested for one to two hour periods. Within those periods, one to three thermal stimuli were presented at randomized times with at least 15 minutes between sequential trials.

Table 1—Mean (±standard error) paw temperature and paw withdrawal latency for all animals tested

<table>
<thead>
<tr>
<th>Behavioral State</th>
<th>Low Ambient Temperature</th>
<th>High Ambient Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Paw Temperature(°C)</td>
<td>Paw Withdrawal Latency(s)</td>
</tr>
<tr>
<td>Waking</td>
<td>31.9 ± 0.5</td>
<td>8.9 ± 0.3</td>
</tr>
<tr>
<td>Drowsy</td>
<td>33.0 ± 0.0</td>
<td>7.0 ± 0.3</td>
</tr>
<tr>
<td>Slow-Wave Sleep</td>
<td>33.2 ± 0.0</td>
<td>6.2 ± 0.2</td>
</tr>
</tbody>
</table>

Analysis

Behavioral observations and/or EEG and EMG recordings were used to determine the behavioral state for each 30 second epoch. The criteria for each state were as follows. A rat was considered to be awake if it had extensor tone in any of its four limbs; during waking, the EEG was desynchronized and low in amplitude while the neck EMG showed both tonic and frequent phasic activity. A drowsy rat had no extensor tone in its four limbs, open eyes and made only gross postural movements; during the drowsy condition, the EEG had short periods of synchronized, high amplitude activity while the neck EMG showed little activity. A rat was considered to be in slow-wave sleep if it lacked extensor tone (sleep posture) and if no non-respiratory movements occurred. During slow-wave sleep, the EEG was synchronized and high in amplitude (≥100 V) while the neck EMG showed minimal tonic activity. Rats were not studied during paradoxical sleep which was marked by “twitching” whiskers, ears and/or extremities, an EEG that resembled the waking pattern, and very flat EMG recordings.

The classifications of behavioral state by behavioral and by physiological measures were the same in more than 88% (n=173 30-second epochs) of the cases. Eight of the misclassifications involved the classification of a period of paradoxical sleep, during which there was no gross twitching, as slow-wave sleep. These results confirm previous findings that the sleep/wake state of a rat can be accurately predicted by behavioral observations alone and suggest that a minority of trials assigned to the slow-wave sleep category were in fact erroneous.

Several testing sessions were required to acquire sufficient samples during waking and slow wave sleep states for each rat. Within each testing session, it was rare to achieve multiple trials for each behavioral state. Because of these considerations, all measurements from a single behavioral state and a single ambient temperature for each animal were used to calculate means and standard errors.

RESULTS

Withdrawal from a Noxious Radiant Heat Stimulus. Rats withdrew from the noxious radiant thermal stimulus when it was applied in each behavioral state tested. In many cases, rats briefly licked the stimulated paw afterwards. The latency to withdraw from a noxious thermal stimulus was dependent on the state of the animal at the time of stimulus onset (see Table 1). During waking, the average paw withdrawal latency (PWL) was 8.9±0.3 seconds at room temperature whereas during slow-wave sleep, the average PWL was 6.2±0.2 seconds. During a drowsy behavioral state, the average PWL was intermediate at 7.0±0.3 seconds. Thus, the slowest responses occurred when the rat was awake at stimulus onset and the most rapid responses occurred
when the rat was in slow-wave sleep. PWLs evoked during waking were greater than those evoked during either drowsy or slow-wave sleep states (ANOVA, p<.001). In addition, PWLs evoked during a drowsy state were significantly greater than those evoked during slow-wave sleep (p<.001).

Since cutaneous temperature increases when rats transit from waking to slow-wave sleep, 15 experiments were performed in order to control for a possible confound of cutaneous temperature. At high (27.5-29.0°C) ambient temperatures, cutaneous temperature does not change between waking and slow-wave sleep states.15 The PWLs evoked during waking, drowsy, and slow-wave sleep states were always less when tested at the high ambient temperature than when tested at the low ambient temperature. As at room temperature, PWLs at the higher ambient temperature were less when evoked during either drowsy or slow-wave sleep states than during waking (ANOVA, p<.001) state and PWLs evoked during slow-wave sleep were less than those evoked during a drowsy state (p<.01).

In some cases (n=9 rats), the temperature of the hindpaw plantar surface was measured with an infrared thermometer, just prior to the application of the heat to the contralateral hindpaw. As shown in Table 1, the mean paw temperature during slow-wave sleep was greater than that during waking at the low but not at the high ambient temperature. There was a significant effect of state (ANOVA, p<.05) and of ambient temperature (p<.002) on PWL but no significant interaction (p>0.2). The cutaneous temperatures during waking and slow-wave sleep were significantly different (p<.05), likely due to the values at the low ambient temperature when the paw temperature during waking averaged 1.2°C less than that during slow-wave sleep. Such a difference in initial paw temperature is likely to produce a change of no more than 0.6 seconds in withdrawal latency,16,17 whereas the difference in latency averaged 1.7 seconds in these nine animals. It should be noted that at the high ambient temperature, where the paw withdrawal latencies differed significantly across state, there were no significant differences in paw temperature. Furthermore, least-squares linear regression analysis revealed that the cutaneous paw temperature was a poor predictor of PWL at either ambient temperature. The average correlation coefficient was -0.31 at high ambient temperatures and -0.44 at low ambient temperatures.

In about half of all trials in which the rat was sleeping at the time of the stimulus onset, the rat was sleeping again within 30 seconds. The average PWL tended to be less when the rat returned quickly (<30 seconds) to slow-wave sleep than when it awoke to an active state.

Withdrawal from a Noxious Laser Heat Stimulus. Rats withdrew from the noxious laser stimulus, using either pulse parameters (see Methods), at latencies of less than 500 ms. The effects of ambient temperature, laser pulse duration, and systemic morphine (0.5—5.0 mg/kg, i.m.) were tested on both the magnitude and the latency of the paw withdrawal. Changing ambient temperature (22 or 27°C) had no effect on either magnitude or latency of the paw withdrawal. Similarly, increasing pulse duration or administering systemic morphine had no effect on response latency (Figure 1). However, the magnitude of the rectified EMG response increased with increasing pulse length and decreased within 10 minutes after morphine administration (Fig. 1).

The magnitude and latency of the average rectified EMG response was compared for laser stimuli applied during slow wave sleep and wake states. In response to the laser stimulus, neither the magnitude nor the latency of withdrawal was different between sleep and waking trials (Figure 2). This result was seen both in rats tested with a 300 ms 2.5 W stimulus (n=8) or in rats tested with a 80 ms 10 W stimulus (n=9). Mean responses from the latter group are shown in Figure 2.

We consistently observed that during sleep, an initial large motor response was often followed by a very short awakening; rats quickly returned to slow-wave sleep (Figure 3). In contrast, when stimulated during waking, a rat typically jerked its head in the direction of the stimulation and then licked the stimulated

Figure 1—Morphine suppression of the paw withdrawal evoked by laser heat. The responses to laser stimulation (2.5 W, 300 ms, the line below each trace) of the paw before (A1-A4) and 12 minutes after (B1-B4) morphine administration (5 mg/kg, i.m.). The magnitude but not the latency (compare A4 and B4) of the response is affected by morphine. The time calibration in B3 applies to traces A1-A3 and B1-B3. The laser pulse in both A4 and B4 marks 300 ms.
These animals often looked around the chamber, sniffed the chamber, changed body position, and explored after withdrawing from the stimulus. We reviewed video tapes to measure the latency to adoption of a sleep posture after laser stimulation. When the laser stimulus was applied to a rat in sleep posture, the rat returned to a sleep posture after an average of 262±177 s (n=6). In contrast, when the laser stimulus was applied during waking, the next sleep posture occurred at a significantly longer latency (Fig. 4, 923±137 s, n=9, t-test, p=0.01).

**DISCUSSION**

Noxious thermal stimulation evokes different reactions when applied during waking or sleep states. The initial motor withdrawal from noxious radiant heat occurs more briskly during slow-wave sleep than during waking. This enhancement of the initial motor response to noxious heat applied during sleep is easily appreciated in teleological terms as a protective reaction during a particularly vulnerable period. Despite a strong initial response, rats that were stimulated during sleep rapidly returned to sleep whereas rats that were stimulated during waking changed their behavior and did not sleep for a prolonged period of time.
These results suggest that noxious stimulation evokes at least two behavioral reactions that are differentially modulated across sleep/wake cycles.

Comparison with Previous Results. The present results are not consistent with a previous study showing that the cat tail flick evoked by radiant heat occurred at a greater latency when elicited during slow-wave sleep than during waking. 33 This discrepancy may be due to a species differences or to differences in the circuitry and modulation of the measured reflex. We favor the latter possibility since several differences between heat-evoked tail and hindpaw withdrawals have previously been reported. 3 Furthermore, the amplitude of the disynaptic jaw-opening reflex (a trigeminal withdrawal reflex) is greater during slow-wave sleep than during waking in cats. 34 Therefore, tail withdrawal may be a special case that is not representative of “nociceptive responsiveness.” Additional studies are needed to clarify these issues.

The Initial Motor Withdrawal

During slow-wave sleep, rats withdrew from noxious radiant heat more quickly than they did when tested during waking. A shorter withdrawal latency to radiant heat is typically interpreted as a reduction in the threshold temperature required for withdrawal. Yet, the magnitude of the laser heat-evoked withdrawals was not different between waking and sleeping. The most likely explanation of these results is that the threshold for evoking a withdrawal changes across behavioral states while the gain of the response is unchanged. In support of this idea, we have observed in preliminary experiments that short laser pulses which produce a sense of warmth but not pain in the investigators, elicit a motor response when applied during sleep but not during waking.

The enhancement of nocifensive withdrawals during sleep relative to waking could be supported by the actions of a number of serotonergic9,18,19 and noradrenergic8,20 neurons which discharge at higher rates during waking than during slow-wave sleep. Locus coeruleus and caudal raphe (magnus, obscurus, and pal- lidus) cells project to the spinal and trigeminal dorsal horns. 21-23 Although the issue has not been directly tested, it is likely that levels of serotonin and norepinephrine within the spinal dorsal horn are greater during waking than during slow-wave sleep as they are in the forebrain. 24-26 Since serotonin and norepinephrine have a primarily inhibitory effect on nociceptive transmission6,7 these monoaminergic bulbospinal neurons may mediate the decrease in the nociceptive threshold observed during sleep.

Within the spinal cord, bulbospinal monoaminergic neurons may inhibit nociceptive transmission at the level of the primary afferent, dorsal horn interneurons and/or the ventral horn. Since motoneuron excitability is the same or less during slow-wave sleep when compared to waking, it is unlikely to contribute to the lowered threshold for initial withdrawals during sleep. 27-29 Instead, it is likely that the responses of dorsal horn sensory neurons are facilitated during slow-wave sleep. Indeed, Satoh et al. 30 demonstrated that the short-latency responses of rostral spinal trigeminal cells to tooth pulp stimulation, at twice threshold intensity, are greater during slow-wave sleep than during quiet waking.

During sleep, there exists a generalized pattern of disinhibition31,32 while during waking, there is widespread suppression of subcortical somatomotor pathways. 31 The increased nociceptive responsiveness observed during slow-wave sleep may reflect a release of spinal somatomotor pathways from supraspinal inhibition which is present during waking. The present results can then be viewed as a sleep-associated disinhibition that allows an animal to maintain vigilance. Unlike during waking, animals can not use visual, olfactory, and auditory clues to avoid noxious stimuli during sleep. Therefore, the disinhibition of nocifensive movements during the sleep state may be a primary method of avoiding injury from noxious stimuli during sleep.

Pain-Evoked Arousal. In the present study, when noxious heat was applied during sleep, rats typically raised their head briefly and then returned to sleep within a short time. Similarly, in humans, cutaneous pain rated at 5.1 on a 10-point visual analog scale and described as “burning “ and “miserable” when applied during waking failed to evoke EEG changes when applied during slow wave sleep. 35 Such a suppression of noxious stimulus-evoked arousals during slow-wave sleep may be an important homeostatic mechanism for ensuring that sufficient time is spent sleeping. 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. A recent anatomical study suggests that the superficial dorsal horn neurons transneuronally labeled from tail withdrawal muscles are rarely retrogradely labeled from thalamus. 36 Therefore, the neurons mediating the motor withdrawal from noxious stimulation may be distinct from those that project rostrally and are involved in producing arousal. In support of such a distinction, stimulation of flexor reflex muscle afferents elicits a flexion reflex during slow-wave sleep at a lower intensity than that required for cortical arousal. 37 It is unlikely that descending monoaminergic cells contribute to the suppression of pain-evoked arousals during sleep. As stated above, the predicted effect of decreasing monoaminergic tone during sleep would be to enhance nociceptive transmission. Instead, it is possible that non-monoaminergic cells in RM suppress transmission of nociceptive information to brain sites.

Figure 4—Mean latencies to resume a sleep posture after laser stimulation. For each animal studied, the latency to resumption of a sleep posture was less when the stimulus was applied when the animal was in a sleep posture than when animal was awake.

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involved in arousal. As mentioned above, we recently demonstrated that RM OFF cells discharge continuously during slow-wave sleep but only intermittently during waking. 38,39 OFF cell activation is hypothesized to inhibit nociceptive transmission within the spinal dorsal horn. 3,4 OFF cell discharge during slow-wave sleep resembles OFF cell discharge after administration of analgesic doses of morphine. 38,39 We have therefore proposed that OFF cell discharge during slow-wave sleep functions to suppress pain-evoked arousals.

CONCLUSIONS

As suggested by Perl, 40 brainstem nociceptive modulatory systems are likely to modulate nociceptive sensitivity in accordance with behavioral state. The present observations suggest that such state-related pain modulation is complex and includes differential effects on distinct nociceptive channels. The lowered withdrawal threshold and the decreased arousal evoked by pain during sleep would have the combined effect of maintaining safety during sleep while also ensuring sufficient sleep time.

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REFERENCES


