Movement-Related Discharge of Ventromedial Medullary Neurons

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Foo, H. and Peggy Mason. Movement-related discharge of ventromedial medullary neurons. J Neurophysiol 93: 873–883, 2005. First published September 22, 2004; doi:10.1152/jn.00750.2004. Studies in anesthetized animals implicate nonserotonergic cells in the ventromedial medulla (VMM) in opioid modulation of nociceptive transmission but do not reveal the conditions that engage VMM cells in unanesthetized rats. The few studies of VMM cells in unanesthetized rats show that VMM cells change their discharge across the sleep-wake cycle and during active movements. Since active movements are more likely to occur during waking than sleep, state-related discharge may in fact represent movement-related discharge. In this study, we recorded the discharge of VMM neurons in unanesthetized, drug-free, freely moving rats and examined whether neuronal activity was related to wake/sleep state, to motor activity, or to both factors. Most cells (45/67) were more active during waking states than sleeping states, 1 cell was more active during sleep states, and the remaining 21 cells did not fire preferentially across the sleep-wake cycle. Most wake-active cells (36/45) showed discharge bursts during movement bursts, and 9/11 wake-active cells were excited by noxious heat and innocuous air puff stimulation. In contrast, few state-independent cells (9/21) showed movement-related bursts in discharge. These results suggest that VMM neurons modulate spinal processes during phasic motor activity.

INTRODUCTION

Brain stem neurons in the ventromedial medulla (VMM) are involved in descending pain modulation. Electrical or chemical stimulation of VMM neurons can either facilitate or suppress spinal pain transmission (Basbaum et al. 1976; Fields et al. 1977; Zhuo and Gebhart 1990, 1992). Conversely, inactivation of VMM attenuates the analgesic effects of systemic morphine and the hyperalgesic effects of opiate withdrawal (Dickenson et al. 1979; Fields et al. 1983b; Kaplan and Fields 1991; Proudfoot and Anderson 1975). In support of the idea that VMM contains pain facilitatory and pain inhibitory neurons, electrophysiological studies using anesthetized rats have identified two types of neurons with opposing responses to painful stimulation and to morphine (Fields et al. 1983a; Heinricher and Drasner 1991; Heinricher et al. 1992, 1994). ON cells are activated by noxious cutaneous heat stimulation and inhibited by morphine and are thought to facilitate pain. OFF cells are inhibited by noxious stimulation, activated by morphine, and considered to inhibit pain. In unanesthetized, freely behaving rats, VMM cells respond to innocuous stimuli such as brush, and even nonsomatosenory stimuli such as clap, in the same direction and to a similar magnitude as they do to noxious stimuli (Leung and Mason 1999; Oliveras et al. 1989, 1990). Thus the response to any stimulus, noxious or non-noxious, can be used to classify VMM cells as ON or OFF. Both ON and OFF cells are nonserotonergic (Auerbach et al. 1985; Gao and Mason 2000; Mason 1997; Potrebic et al. 1994).

In unanesthetized rats, VMM cells discharge in relation to phasic movements of the head, body, and proximal limbs (Leung and Mason 1999; Oliveras et al. 1990). Since VMM cells fail to respond to proprioceptive inputs (Casey 1969; Moolenaar et al. 1976), movement-related discharge likely reflects corollary discharge from a motor command center. Alternatively, since the spontaneous activity of nonserotonergic VMM cells also varies across the sleep-wake cycle (Leung and Mason 1999), discharge that seems to be movement-related may in fact represent state-related discharge. Most ON cells show a wake-active pattern of discharge, with bursts of activity during waking and low or no activity during slow wave sleep (Leung and Mason 1999). On cells also show an increase in discharge during phasic movements. In contrast, OFF cells are sleep-active, displaying continuous activity during slow wave sleep and only sporadic activity during waking; OFF cells decrease their firing during phasic movements. Motor activity is highly correlated with state as generalized muscle tone is higher and movements occur more frequently during waking than during sleep. Thus, although the sleep-wake activity patterns of ON and OFF cells suggest a state-related input, this state-related discharge may actually represent movement-related discharge. These experiments are designed to differentiate between VMM discharge related to phasic axial movements and that related to sleep-wake state.

METHODS

Surgical preparation

All procedures were reviewed and approved by the University of Chicago Institutional Animal Care and Use Committee. Male Sprague-Dawley rats (350–450 g; Charles River) were anesthetized with 1.8–2.0% halothane via a nose cone. They were placed in a standard stereotaxic apparatus and on top of a water-perfused heating pad that maintained their core temperature at 37.0–37.5°C. Stainless steel screws were placed in the frontal and parietal bones for differential EEG recording, and stranded stainless steel microwires were sutured into the nuchal muscles, the bulbospinous muscles at the base of the penis, and the biceps femoris muscles of the right hind paw for EMG recording. The EEG and EMG leads were attached to Omnetics microconnector (Minneapolis, MN) and affixed to the skull. A small craniotomy was performed, and a threaded microdrive base with a guide tube was implanted in the area overlying VMM (~11.3 from bregma, at midline) for the introduction of tungsten recording electrodes (5 MΩ, A-M Systems, Carlsborg, WA).

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Apparatus

The test cage was a Plexiglas box (25 × 45 cm, 35 cm height) with a wire-mesh floor. It stood 28 cm above the base of the testing chamber. A modified Hargreaves apparatus (Hargreaves et al. 1988) was located underneath the test cage. This apparatus consisted of a focused light bulb that produced radiant heat and was used to deliver noxious thermal stimulation to the hind paw. The heat intensity was set to elicit paw withdrawal latencies of 4–5 s. The heat stimulus terminated on paw withdrawal or when a maximum duration of 8 s was reached. Air puff was delivered at a rate of 5 liters/min via tubing attached to a plastic pipette (inner tip diameter = 2 mm).

Experimental protocol

The rats were allowed ≥1 wk to recover from surgery. After recovery from surgery, they were familiarized to the handling procedure on recording days and to the test apparatus. On recording days, the rat was held gently while a microdrive-electrode assembly was connected to the threaded base and a flexible commutator cable was attached to the microconnector on the rat’s head. The rat was placed into the test cage where he was able to move freely and had free access to food and water. The recording electrodes were advanced manually into the VMM region until a unit was isolated. All cells discharged irregularly and/or at high rates and were therefore likely nonserotonergic; this was verified using a previously described algorithm (Mason 1997). The rat’s behavior was continuously monitored and videotaped with cameras located at the front and sides of the cage. The video and physiological recordings were synchronized.

The cellular and behavioral responses to noxious thermal stimulation of the hind paw were determined 2–10 times, with an interstimulus interval of >5 min. Air puff stimulation was delivered to the head or back (see lines under unit trace in Figs. 3A and 5A). Air puff durations varied but averaged 5.3 ± 0.4 s.

The recording depth for every unit was noted. At the end of each recording session, the cable and the microdrive-electrode assembly were removed. The rats were tested once a week. On the last test day, the final recording site from each animal was marked by applying 20-μA anodal current for 4 min. All other recording sites from each animal were calculated from the marked site.

Data acquisition

A preamplifier within the commutator cable amplified the EEG and EMG signals fivefold. These signals were further amplified 10,000-fold by a differential AC amplifier (A-M Systems, Carlsborg, WA) and digitized at 1 kHz by a Power1401 interface (Cambridge Electronic Design, Cambridge, UK). The preamplifier buffered the unit signal at unity gain. This signal was amplified 10,000-fold by an AC differential amplifier (Warner Instruments, Hamden, PA) and digitized at 20 kHz by the Power1401 interface. Extracellular recordings were taken from all units that provided stable recordings (see Figs. 3A, 4E, and 5E). For each isolated unit, an amplitude threshold was set using Spike2 acquisition software (CED, Cambridge, UK). When the signal crossed this threshold, the time of that crossing was stored. In addition, 46 digitized points of the waveform were saved: 21 points before and 25 points after threshold crossing. Individual waveforms were reviewed off-line, sorted according to amplitude and shape, and assigned to a particular unit using a template-matching algorithm provided by Spike2. Most cells were recorded singly (n = 40) or in pairs (n = 24). The remaining three cells were recorded together.

Histological processing

All rats were overdosed with sodium pentobarbital (ip) and perfused with a fixative containing 10% formalin in 0.1 M PBS (pH 7.4). The brain stem was removed and placed in 30% sucrose. Serial coronal sections (50 μm) were cut on a freezing microtome, mounted on slides, and stained with cresyl violet. The marked recording sites were examined microscopically and plotted onto standard sections.

Behavioral classification

Behavioral observations and/or EEG and EMG measures were used to determine wake and sleep states. State assignments were made continuously with no minimum bout length. Four states were recognized: active wake, quiet wake, slow-wave sleep, and paradoxical sleep. The rat was considered to be in active wake if he was engaged in active behaviors such as grooming, eating, drinking, urinating, defecating, or exploring. During such behaviors, the EEG was desynchronized and low in amplitude, while the EMGs showed both tonic and phasic activity. The rat was judged to be in quiet wake when he was sitting quietly with occasional head movements and postural adjustments. The EMG showed low tonic activity except during the brief isolated moves. The EEG was desynchronized with short periods of synchronized, high-amplitude activity. Brief arousals during which the rat made slight postural adjustments before returning to sleep were also classified as quiet wake. During these microarousals, the EEG was desynchronized, and the nuchal and/or leg EMG showed a short burst of phasic activity. The rat was considered to be in slow wave sleep when he adopted a sleep posture (van Betteray et al. 1991; Van Twyver 1969) and displayed primarily respiratory-related movements. In this sleep state, the EEG was synchronized and high in amplitude, while all EMG measures showed low tonic activity. During paradoxical sleep, the EEG was desynchronized and low in amplitude. Behaviorally, we observed and recorded on video tape “twitching” of whiskers, ear, and/or paws. Such activity in distal musculature was typically not evident in the nuchal EMG, which was low to very low in amplitude during paradoxical sleep. All wake and sleep epochs were included in the analyses, irrespective of bout length. A minimum bout duration was not set because it is l) an arbitrary standard and 2) likely to exclude very short-duration microarousals. To include only cells that were recorded across wake and sleep states, a minimum number of quiet wake and slow wave sleep bouts was set.

Cellular activity during sleep-wake states

Only cells (n = 67) recorded across at least five bouts each of quiet wake and slow wave sleep were used. For each cell, mean discharge rates for each bout in each state were calculated, and cell averages for each state were calculated by averaging across all bouts within a state. The mean discharge rates of the states for each cell were compared using ANOVAs, with significance set at the 0.05 level. Cells were then classified as wake-active, sleep-active, or state-independent. Population mean discharge rates for each of the four states were computed and tested for significance with ANOVAs (α = 0.05).

Movement-related cellular discharge

To determine the relationship between VMM cell activity and movements, the nuchal EMG was used as a measure of axial and proximal limb movements (see DISCUSSION). Our aim was to assess the relationship between cellular and muscular activity in two ways: one general and one related to bursts. The first type of analysis examined the relationship between cellular discharge and general motor activity using linear regressions in a method very similar to that used by Lee et al. (2004). For all analyses, the nuchal EMG was full wave rectified. Cellular discharge and integrated nuchal EMG activity were calculated for each behavioral state epoch recorded. Linear regressions of cellular discharge on integrated nuchal EMG activity were conducted to assess whether neuronal activity was predicted by this measure of generalized motor activity. This measure was considered to indicate...
generalized motor activity because all nuchal EMG activity was used, without differentiating between tonic and phasic motor activity.

The second analysis examined more specifically the relationship between cellular discharge and phasic motor activity. For each animal on each test day, a movement threshold ($x = 2\sigma$) that occurred within 1 s of each other were grouped together within a movement burst. The average burst duration was 22.2 s and ranged from 1.0 to 892.6 s. For each cell, the proportion of all spikes that occurred during movement bursts was compared with the proportion of recording time that was classified as part of a movement burst (proportional $t$-test, $\alpha = 0.05$). A significant difference between the two proportions indicated that cellular discharge was either positively or negatively related to movement bursts. In addition, the spike/time ratio during movement bursts was compared with the spike/time ratio outside of movement bursts to determine the likelihood of firing during movement bursts.

A final analysis was used to quantify the timing relationship between discharge and phasic movements. In selected cells, we examined the timing of cellular discharge relative to the onset of movements (see Figs. 3–5). The number of spikes that occurred 50 s before and after each movement that exceeded the set threshold was calculated in 1-s bins. These values were plotted (black line) against the number of spikes that would have occurred in each bin if the same number of total spikes were equally spaced across time (dashed red line). The two distributions were compared with a ranked sum analysis.

**Cellular responses to external stimulation**

To compare the results with previous work in the VMM, selected cells ($n = 14$) were tested for their responses to external stimulation. Noxious heat stimulation was applied to the hind paw using a modified Hargreaves apparatus (Hargreaves et al. 1988) and air-puff was applied to the face. Individual histograms (bin size: 50 ms) were used to determine the cellular responses to the external stimuli. For each cell, the average discharge preceding the heat (10 s) or air-puff (5 s) was compared ($t$-test, $\alpha = 0.05$) to the average discharge after the onset of the stimulus (10 s for heat and 5 s for air-puff). The sleep-active cell was tested for its response to noxious ear pinch.

**RESULTS**

**Cellular location and characterization**

All recorded cells ($n = 67$) were located in the midline raphe or adjacent nucleus reticularis magnocellularis (Fig. 1). Most cells ($n = 60$) discharged in bursts with a mean CV of the interspike interval ($CV_{ISI}$) of $4.67 \pm 0.35$ and a mean discharge rate of $8.7 \pm 1.3$ Hz. A small number of cells ($n = 7$) did not burst ($CV_{ISI} < 1$) and had a mean $CV_{ISI}$ of $0.72 \pm 0.05$ and a mean discharge rate of $32.7 \pm 4.5$ Hz (minimum: 16.6 Hz). Thus none of the recorded cells displayed the slow and steady discharge characteristic of serotonergic cells in anesthetized and unanesthetized animals (Auerbach et al. 1985; Fornal et al. 1985; Heym et al. 1982; Mason 1997).

**Frequency and mean duration of wake and sleep states**

Overall, the rats spent 45% of the recording time awake and the remainder in sleep. Slightly less time was spent in active waking (20.7%) than in quiet waking (24.3%). There were fewer active wake ($n = 356$) than quiet wake bouts ($n = 2999$), but active wake bouts were longer in duration than quiet wake

![FIG. 1. Distribution of recording sites of ventromedial medullary (VMM) wake-active (△), sleep-active (●), and state-independent (+) cells. The numbers indicate the anterior-posterior level from bregma for each section.](http://jn.physiology.org/)

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bouts (Fig. 2). More than 75% of active wake bouts were >10 s, with a mean bout length >2 min (167.2 ± 13.4 s). Quiet wake bouts were shorter in duration (mean = 23.2 ± 1.0 s, Fig. 2), with most bouts lasting <10 s. Less sleep time was spent in slow wave sleep (23.4%) than in paradoxical sleep (31.6%). Slow wave sleep bouts occurred more frequently (n = 2,879) than paradoxical sleep bouts (n = 1,270). The slow wave sleep bouts were short in duration: one-half of the bouts were <20 s (mean: 23.4 ± 0.4 s). Paradoxical sleep bouts were longer in duration (mean: 71.4 ± 2.0 s; Fig. 2), with most bouts extending beyond 20 s.

**Relationship between cellular activity and wake/sleep states**

Forty-six of the 67 cells exhibited significant changes in spontaneous discharge across wake and sleep, with 45 cells displaying a wake-active pattern (Fig. 3) and 1 cell displaying a sleep-active pattern of activity. The remaining 21 cells displayed discharge that did not differ significantly across wake-sleep states (Figs. 4 and 5).

**WAKE-ACTIVE CELLS.** The population mean discharge rate of wake-active cells was higher during quiet wake (11.9 ± 1.9 Hz) than during slow wave sleep (6.6 ± 1.5 Hz; ANOVA, P < 0.05). Thirty cells were also recorded during active wake. The population mean discharge rate of these cells during active wake (13.1 ± 1.6 Hz) was not significantly different from their population mean discharge rate during quiet wake (10.7 ± 2.0 Hz). The discharge pattern of individual cells reflected the overall population pattern: most cells (n = 26) discharged at the same rate across the two wake states; three cells discharged at a higher rate during active wake; and one cell discharged at a higher rate during quiet wake. For the 40 wake-active neurons recorded during paradoxical sleep, their population mean discharge rate during slow wave sleep (7.2 ± 1.7 Hz) was the same as their population mean discharge rate during paradoxical sleep (6.6 ± 1.5 Hz). This pattern was also observed when individual cells were analyzed with most cells (33/40) displaying equal discharge across the two sleep states. For the remaining cells, five showed higher activity during paradoxical sleep than slow wave sleep, and two showed higher activity during slow wave sleep.

**SLEEP-ACTIVE.** The sleep-active cell had a mean discharge rate of 13.5 Hz during active wake, 25.8 Hz during quiet wake, and 51.1 Hz during paradoxical sleep (data not shown). The discharge rates during waking were significantly less than the discharge rates during sleep (P < 0.05), with no reliable differences within wake or sleep substates.
STATE-INDEPENDENT. For the 21 cells with state-independent discharge, the population mean discharge rates were 18.3 ± 4.3 Hz during quiet wake and 18.0 ± 4.4 Hz during slow wave sleep. For the 11 state-independent cells recorded during active wake, their population mean discharge rates during active wake (14.3 ± 4.1 Hz) and quiet wake (14.0 ± 4.7 Hz) were the same. Additionally, when analyzed individually across all the bouts recorded for each cell, none of the cells differed significantly in their discharge rates across the two wake states. All 21 cells were recorded during paradoxical sleep, with no
FIG. 5. Discharge of this cell was unrelated to wake and sleep states, generalized motor activity, or movement bursts. Conventions are as in Fig. 3. A: innocuous air-puff stimulations (lines under unit trace) evoked motor responses without changes in cellular activity. B and C: activation of nuchal muscles was not associated with changes in cellular discharge. D: number of spikes that occurred near or at movement onset (black line) was not different from the number of spikes that would have occurred if they were uniformly distributed across time (dashed red lines; \( P = 0.8 \)). E: stable recordings were obtained from this cell across wake-sleep states and movements.
difference in their population mean discharge rates between sleep states (slow wave sleep = 18.0 ± 4.4 Hz; paradoxical sleep = 18.0 ± 4.4 Hz). Most cells (n = 19) showed the same discharge rate across both sleep states, with only two cells discharging at a higher rate during paradoxical sleep.

Relationship between cellular and motor activity

As described in methods, integrated nuchal EMG for each wake and sleep epoch was used as a measure of generalized motor activity. This measure increased during waking and reflected an overall increase in motor activity such as exploratory head movements and locomotion. For all wake and sleep epochs of each cell, integrated motor activity and neural discharge were analyzed using linear regression to determine if cellular discharge was predicted by integrated motor activity. In addition, we tested for a relationship between cellular discharge and bursts of phasic movements. A movement burst was defined as a grouping of at least two suprathreshold (see Methods) neck movements that occurred within 1 s of each other. The proportion of spikes occurring during movement bursts was compared with the proportion of time that was included in the movement bursts using a proportional t-test (see Methods). If spike probability were independent of the timing of movement bursts, these proportions would be equal, whereas if spikes were more likely to occur during bursts, the proportion of spikes would be greater than the proportion of time, and so on. The spike:time ratios during movement bursts were compared between cell types (see Methods). A spike:time ratio of unity would indicate that spikes occurred independently of movement timing. A ratio >1 would indicate an increased probability, and a ratio <1 would indicate a decreased probability of spikes occurring during movement bursts.

Wake-active cells. As integrated motor activity increased, the cellular discharge of 34/45 wake- active cells increased. For instance, as seen in Fig. 3A, integrated muscle activity and cellular discharge were both higher during waking than during sleep. However, the $r^2$ values for the significant responses averaged 0.21, and 32/34 had $r^2$ values of <0.38. Thus integrated muscle activity was a poor predictor of cellular discharge.

For the majority of wake-active cells (36/45), cellular discharge was, on average, 3.2 times more likely to occur during bursts (1 s) than if spikes were evenly distributed across time (Fig. 3D, black line) than would be predicted if the spikes were uniformly distributed across time (dashed red line). It should be noted that the period of preferred discharge is distributed both before and after movement initiation. A ranked sum analysis of the actual and predicted distributions indicated that they were significantly different ($P < 0.05$). In the remaining nine wake-active cells, cellular discharge was slightly less likely (average = 0.78 times) to occur during movement bursts for six cells and was just as likely to occur during movement bursts as outside of movement bursts for three cells.

Sleep-active cell. This cell’s discharge was inversely related to integrated muscle activity and movement bursts. During periods of high motor activity, cellular discharge was low. However, the linear regression function of its discharge on integrated motor activity had an $r^2$ value of only 0.11. The decrease in discharge associated with movement was more evident as a 2.8-fold reduction in the number of spikes during movement bursts. The timing of the decrease in discharge occurred in close proximity to movement onset: although not significant, there was a tendency ($P = 0.15$) that fewer spikes occurred near or at the onset of movements than would have occurred if the spikes were evenly distributed across time.

State-independent cells. Integrated motor activity was a very poor predictor of cellular discharge for 6/21 of the state-independent cells, with $r^2$ values that averaged 0.10 and were mostly <0.09 (5/6). On average, nine state-independent cells were more likely to fire during movement bursts than in the absence of phasic movements (ratio of 1.8). An example of a state-independent cell with discharge related to movement bursts is shown in Fig. 4, A–C. As can be seen from this figure, the bursts in cellular discharge corresponded well with bursts in EMG activity. For this cell, the number of spikes occurring at or near movement onset (black line) was significantly higher than if spikes were evenly distributed across time (dash red line; $P < 0.05$).

Five state-independent cells were less likely to fire during phasic movements (ratio = 0.58). The remaining seven state-independent cells showed discharge that was not associated with movement burst. An example of a state- and motor activity-independent cell is shown in Fig. 5. The discharge of this cell was unrelated to state, integrated motor activity, or movement bursts. Movements that occurred spontaneously or evoked by innocuous air-puff stimulation (lines under trace) were not accompanied by changes in cellular discharge. The number of spikes that occurred at or near the onset of movement bursts (black line) was the same as the number that would have occurred if the spikes were evenly spaced across time (dash red line; Fig. 5D; $P = 0.8$).

Cellular response to external stimulation

Fourteen cells were tested for responsiveness to noxious radiant heat and innocuous air puff. Nine of the 11 wake-active movement-related cells tested were excited by both noxious radiant heat and innocuous air puff stimulation. The remaining two cells were either excited or inhibited by noxious heat, and both were unresponsive to air-puff stimulation. One wake-active movement-unrelated cell was tested and did not respond to either noxious heat or air puff stimulation. Of two state- and movement-independent cells tested, one cell was excited by
both types of external stimulation, whereas the other cell was inhibited by heat and unresponsive to air puff stimulation. The sleep-active cell was inhibited by noxious ear pinch.

**DISCUSSION**

**Sleep-wake behavior**

Not surprisingly, there were relatively few active wake bouts as recordings were taken during the light cycle when rats generally sleep. Although few in number, active wake bouts tended to be lengthy, because rats would typically chain behaviors together in a series, for example, foraging followed by eating, and then grooming. There were many more quiet wake bouts, but they were short in duration, primarily because brief awakenings or microarousals in between sleep bouts occurred often.

The rats spent 55% of the recording time asleep, much less than expected from the light part of their cycle (Van Twyver 1969). It is likely that this is due to several factors that either delayed sleep onset or interrupted sleep. After placement in the test cage, the rat usually explored his environment before settling down to sleep. Moreover, sleep was also delayed or disrupted by movement of the microdrive-electrodes assembly during the cell search and isolation process. On occasion, we observed that rats found it difficult to acquire a comfortable sleep posture or were awoken by the cable or by unintended noises in the room. The combined effects of delayed sleep onset and interrupted sleep might have also facilitated rats entering paradoxical sleep, explaining why more sleep time was spent in this state. Slow wave sleep bouts were typically lengthy, because rats would typically chain behaviors together in a series, for example, foraging followed by eating, and then grooming. There were many more quiet wake bouts, but they were short in duration, primarily because brief awakenings or microarousals in between sleep bouts occurred often.

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The present results have confirmed that VMM contains nonserotonergic cells with wake- and sleep-active patterns of discharge (Cespuglio et al. 1981; Fornal et al. 1985; Kanamori et al. 1980; Leung and Mason 1999; Sheu et al. 1974; Steriade et al. 1984). In a previous study, using similar but not identical methods, we observed the same types of wake-sleep related discharge patterns but in different proportions ($\chi^2 < 0.001$; Leung and Mason 1999). Previously, we found that 66% of the recorded cells were wake-active, 25% were sleep-active, and 9% were state-independent ($n = 21, 8, \text{ and } 3$, respectively). In this study, 67% of the recorded cells displayed a wake-active pattern of discharge, only 2% exhibited sleep-active pattern of discharge, and 31% showed equal discharge across sleep-wake states ($n = 45, 1, \text{ and } 21$, respectively). Thus we now find a higher proportion of state-independent cells and a lower proportion of sleep-active cells. This discrepancy may be due to differences in experimental protocols. Here, units were isolated while the animals were awake, a condition that favors the detection of wake-active cells. In our past study, most units were isolated while the animals were anesthetized. It has been suggested that the physiological characteristics of VMM nonserotonergic cells are modified by anesthesia, such that OFF cells are inactive in the unanesthetized condition and become active under anesthesia (Oliveras et al. 1991a,b). This evidence of a sleep-active cell that is inhibited by noxious ear pinch in a drug-free, freely behaving rat confirms our previous observation (Leung and Mason 1999) that OFF cells are active under normal physiological conditions. Yet, it is likely that OFF cells are much less active in the awake, drug-free, freely behaving rat than in the anesthetized rat.

**Cellular discharge and wake/sleep state**

The rats spent 55% of the recording time asleep, much less than expected from the light part of their cycle (Van Twyver 1969). It is likely that this is due to several factors that either delayed sleep onset or interrupted sleep. After placement in the test cage, the rat usually explored his environment before settling down to sleep. Moreover, sleep was also delayed or disrupted by movement of the microdrive-electrodes assembly during the cell search and isolation process. On occasion, we observed that rats found it difficult to acquire a comfortable sleep posture or were awoken by the cable or by unintended noises in the room. The combined effects of delayed sleep onset and interrupted sleep might have also facilitated rats entering paradoxical sleep, explaining why more sleep time was spent in this state. Slow wave sleep bouts were typically interrupted by very short microarousals that were considered quiet wake (see Fig. 3A). Thus even though slow wave sleep bouts seem to be short in duration, the animals often spent tens of minutes mostly sleeping.

We previously reported that bursts of discharge in wake-active cells corresponded with isolated head and body movements, whereas the discharge of sleep-active cells was usually inhibited during spontaneous movements (Leung and Mason 1999). Similarly anecdotal observations have been noted by others (Fornal et al. 1985; Oliveras et al. 1989, 1990; Steriade et al. 1984). For example, Oliveras et al. (1989, 1990) observed neurons that discharged during neck and generalized body movements, including cells that responded to external stimulation.

These results provide the first quantitative analysis of discharge in VMM cells as it relates to phasic bursts of neck movements. This relationship was particularly evident among wake-active cells, with >90% of these cells displaying discharge that was associated with bursts of activity in the nuchal muscles. Such an association was less prevalent, but was observed, in 57% of the state-independent cells. The nuchal muscles are activated by most, if not all, postural and head movements (Abrahams et al. 1993; Richmond et al. 1992; Thomson et al. 1994, 1996), locomotion (Sinnammon and Polania 1997), scratching (Carlson-Kuhta and Smith 1994), and even during gaze shifts (Grantyn and Berthoz 1987; Kitama et al. 1995; Roucoux et al. 1989). Stimulation within the dorsal medullary reticular formation elicits limb movements, including locomotion, that are always accompanied by head movements and neck EMG activation (Drew and Rossignol 1990a,b). Thus, although the neck muscles are not activated in all movements, their activation is associated with most axial and locomotive movements.

As mentioned in the Introduction, since VMM cells do not respond to proprioceptive inputs (Casey 1969; Moolenaar et al. 1976), it is likely that movement-related discharge in VMM cells arises from a corollary discharge input from motor command centers. In agreement with this idea, VMM discharge associated with movements were centered about the start of the movement, occurring in the seconds both before and after muscle activation.

We previously reported that bursts of discharge in wake-active cells corresponded with isolated head and body movements, whereas the discharge of sleep-active cells was usually inhibited during spontaneous movements (Leung and Mason 1999). Similarly anecdotal observations have been noted by others (Fornal et al. 1985; Oliveras et al. 1989, 1990; Steriade et al. 1984). For example, Oliveras et al. (1989, 1990) observed neurons that discharged during neck and generalized body movements, including cells that responded to external stimulation.

These results provide the first quantitative analysis of discharge in VMM cells as it relates to phasic bursts of neck movements. This relationship was particularly evident among wake-active cells, with >90% of these cells displaying discharge that was associated with bursts of activity in the nuchal muscles. Such an association was less prevalent, but was observed, in 57% of the state-independent cells. The nuchal muscles are activated by most, if not all, postural and head movements (Abrahams et al. 1993; Richmond et al. 1992; Thomson et al. 1994, 1996), locomotion (Sinnammon and Polania 1997), scratching (Carlson-Kuhta and Smith 1994), and even during gaze shifts (Grantyn and Berthoz 1987; Kitama et al. 1995; Roucoux et al. 1989). Stimulation within the dorsal medullary reticular formation elicits limb movements, including locomotion, that are always accompanied by head movements and neck EMG activation (Drew and Rossignol 1990a,b). Thus, although the neck muscles are not activated in all movements, their activation is associated with most axial and locomotive movements.

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**Cellular responses to external stimulation**

In agreement with our previous finding (Leung and Mason 1999), nearly all (10/12) wake-active cells were excited by noxious thermal stimulation and nearly all cells excited by noxious heat stimulation were wake-active (10/11). Most (9/11) of the wake-active cells excited by noxious heat were also excited by innocuous air puff stimulation. Finally, all of the wake-active cells excited by noxious heat had spontaneous discharge that was movement-related. These findings confirm that most wake-active cells are excited by noxious and innocuous stimuli (Leung and Mason 1999). In our previous study, we also tested the response of such cells to noxious stimulation during anesthesia and observed that these cells were on cells.
Therefore the wake-active cells excited by noxious stimulation and with movement-related discharge recorded in this study \((n = 10)\) are likely to correspond to ON cells. Using similar reasoning, it is likely that the sleep-active cell, inhibited by noxious stimulation and with discharge inversely related to movement bursts, corresponds to an OFF cell. There is insufficient data to suggest a classification for the remaining cells \((n = 56)\).

**Functional implications**

VMM cells project to the ventral horn and motor-related brain stem areas (Masson et al. 1991; Mileykovskiy et al. 2002; Sasek et al. 1990). In this regard, it is interesting to note that microinjection of either kainate or morphine into VMM of the awake rat evokes a decrease in locomotion that is at least in some cases a total immobility (Morgan and Whitney 2000). The decrease in open field exploration evoked by kainate is associated with VMMON cell bursts. Since a moving rat is more tempting to attract the attention of a predator than an immobile rat, helping an animal better react to natural dangers.

VMM nonserotonergic cells are suitable candidates to modulate spinal processes during phasic movements. It is unlikely that these cells modulate specific muscle groups, since movement-related discharge was associated with movements of the head, body, and proximal limbs. In anesthetized rats, VMMON cell bursts. Since a moving rat is more likely to attract the attention of a predator than an immobile rat, movement-associated activity in wake-active ON cells, and the consequent facilitation of stimulus-evoked withdrawals, may help an animal better react to natural dangers.

**References**


