Sleep and Behavior in Rats With Pontine Lesions Producing REM Without Atonia

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Pontine tegmental lesions can eliminate the atonia of rapid eye movement sleep (REM) and allow animals to exhibit overt behavior at the same time that other electrophysiological signs of REM are intact. We produced electrolytic lesions in the reticularis pontis oralis (RPO) and reticularis pontis caudalis (RPC) in rats and observed them for behavior during REM without atonia (REM-A) and for alterations in sleep and waking compared to normal rats. Relatively small unilateral lesions were sufficient to remove the atonia of REM, whereas larger, bilateral lesions were required to release overt behavior during REM-A. Lesions in both RPO and RPC were capable of releasing elaborate behaviors, including full locomotion, during REM-A. The results are discussed with respect to their importance for understanding the neural activity of REM and with respect to REM-A being a model for REM Behavior Disorder (RBD) in humans.

CURRENT CLAIM: REM without atonia in rats may be a useful model for REM Behavior Disorder.

Neural structures necessary for the skeletal muscle atonia of rapid eye movement sleep (REM) are located in the dorsolateral pontine tegmentum (Chase and Morales, 1990). Relatively selective lesions in this vicinity can eliminate the atonia of REM while leaving other identifying features of REM intact (Jouvet and Delorme, 1965; Henley and Morrison, 1974; Hendricks et al., 1982). These include activated EEG, ponto-geniculo-occipital (PGO) waves, increased brain temperature, relaxed nictitating membranes (in cats), and clearly observable rapid eye movements if the head is restrained (Henley and Morrison, 1974). As with normal REM, episodes of REM without atonia (REM-A) follow non-REM. Depending on lesion site and size, the overt behavior released ranges from simple head raising to quadrapedal locomotion. In cats, the head often moves in ways that resemble searching (Henley and Morrison, 1974). In fact, the REM-A preparation in cats was instrumental in the identification of REM behavior disorder in humans (Schenck et al., 1986) (Video 1).

Most work on REM-A has been conducted in cats; however, brief reports have indicated that rats show many similar behaviors (Mouret et al., 1967; Mirmiran, 1983). We have begun to develop and extend the REM-A model in rats in order to address questions relating to the neural basis of REM generation and perhaps to REM behavior disorder in humans. As a first step in this process, we have examined the effects of a series of electrolytic lesions placed in the pons on sleep and on the atonia of REM.

METHODS

The subjects were 27 male Sprague-Dawley strain rats approximately 90 days old at the time of surgery. The rats were implanted with skull screws for recording the electroencephalogram (EEG) and with stainless steel wire electrodes sutured to the olfactory bulb and hippocampal theta activity. Leads from the recording electrodes were routed to a nine-pin miniature plug that mates to one attached to a recording cable.

Seventeen rats received bilateral electrolytic lesions in the pons at the time of implantation, and one received only a unilateral lesion. The remaining 10 did not receive lesion and served as controls. For production of electrolytic lesions, a stainless steel electrode, insulated except for 1.0 mm at the tip, was lowered to the appropriate coordinates, and a current ranging from 0.5-14 sec and 0.5-12.0 µA was passed. Electrolytic lesions were produced in nucleus reticularis pontis oralis (RPO) at coordinates P0.9, ML±1.1, DV -6.5, and/or nucleus reticularis pontis caudalis (RPC), coordinates P1.5, ML±1.5, DV -6.8.

All surgical procedures were performed stereotaxically under aseptic conditions. Ketamine (85 mg/kg) and Xylazine (12 mg/kg) were administered intraperitoneally for anesthesia. Buprenorphine (0.6 mg/kg) was administered for potential post-operative pain. The rats were allowed seven days to recover prior to beginning sleep recording.

Polygraphic studies were conducted on one to three days following recovery between 10 a.m. and 4 p.m. Eastern time. Wakefulness, non-REM (NREM), transition and REM-A were determined by trained observers. The parameters examined were total recording period (TRP), time spent asleep (TSA), sleep efficiency (TSA/TRP), total REM or REM-A time, number and mean duration of wakefulness, NREM, transition and REM or REM-A episodes.

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Electrographic and behavioral criteria for distinguishing behavioral states were as follows:

1. Wakefulness: body movements smoothly performed; low voltage fast EEG (up to 80 µV and 40 Hz); high amplitude tonic EMG and phasic EMG bursts (>2 x baseline within 1 sec);

2. NREM: EEG (2-10 Hz, 200-400 µV), continuing into a pattern of spindles (5-15 Hz) interspersed with slow waves; EMG diminished in amplitude and no intruding gross bodily movements or EEG desynchronization;

3. Transition: prior to REM; sleep spindles (mean frequency 12.5 Hz) present with low frequency theta (5.4 Hz), no intruding gross bodily movements or EEG desynchronization;

4. REM: onset of REM occurs immediately following the last sleep spindle (EMG activity either absent or disappears within a few seconds in normal rats while lesioned rats display REM-A); low voltage fast EEG with theta present; movements in REM-A were ataxic with sudden collapses of postural support while locomoting and then continued walking; attempts to rear on the walls and then falling backwards (not seen in wakefulness); eyes were partially closed.

Behavioral observation began on the first day post-surgery and prior to polygraphic recording. Following nomenclature developed through work with cats (Sanford et al., 1994), behaviors released during REM-A were categorized into one of five behavioral groups distinguished by the level and type of behavior exhibited by the animals. Group I is characterized by increased nuchal muscle tone, vigorous truncal and proximal limb movements, yet no head-raising or coordinated movements. Group II adds head-lifting, while animals displaying Group II behavior display coordinated movements involving the head, neck and forelimbs that resemble orienting (OR) or searching with the snout twitching vigorously and eyes closed. Thus, Group II consists of a release of nuchal tone and the ability to display seemingly directed movements as opposed to mere head lifting but without the inclusion of hind limb support and locomotion. Group III behavior in cats is comprised of violent, phasic behavior resembling pouncing with the forelimbs as if in a predatory attack: such behavior is interspersed with times of quiet staring or searching movements. Group IV involves full quadrupedal locomotion and the behaviors of Group II. It can include Group III behavior as well.

The rats were assigned to categories based on post-lesion behaviors, with the observers blind to the intentioned lesion site and condition. In addition, non-lesioned rats were included for comparison of the various sleep parameters measured.

Upon completion of the experiment, the rats were overdosed with sodium pentobarbital and perfused intracardially with 0.9% saline followed by 10% formalin. The brains were processed histologically to determine lesion placements. For this purpose the brains were embedded in celloidin; 40 µm slices were made through the areas of interest and the sections were stained with cresyl violet.

RESULTS

Sleep

Figure 1 demonstrates EEG and EMG recordings from a normal rat and a lesioned rat. Descriptive sleep parameters for individual animals are presented in Table 1. Given the limited number of animals in each behavioral category statistical analyses were not performed. However, REM-A episode duration in lesioned rats appeared to be decidedly shorter after some lesions (Note in particular Ra9 and CC9).
Table 1. Sleep Parameters in Rats Exhibiting REM-A and in Non-lesioned Rats

<table>
<thead>
<tr>
<th>GROUP</th>
<th>ANIMAL</th>
<th>REM/REM-A TOTAL</th>
<th>REM/REM-A DURATION</th>
<th>REM/REM-A COUNT</th>
<th>REM/REM-A PERCENT</th>
<th>TIME SPENT ASLEEP</th>
<th>SLEEP EFFICIENCY</th>
<th>NUMBER OF DAYS</th>
</tr>
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<tr>
<td>NORMAL (n=10)</td>
<td></td>
<td>Avg</td>
<td>SD</td>
<td>Avg</td>
<td>SD</td>
<td>Avg</td>
<td>SD</td>
<td>Avg</td>
</tr>
<tr>
<td>I</td>
<td>CC3</td>
<td>13.34</td>
<td>4.06</td>
<td>1.32</td>
<td>0.12</td>
<td>10.33</td>
<td>4.16</td>
<td>9.37</td>
</tr>
<tr>
<td>I</td>
<td>Ra2</td>
<td>37.49</td>
<td>12.60</td>
<td>2.02</td>
<td>0.60</td>
<td>18.50</td>
<td>0.71</td>
<td>20.17</td>
</tr>
<tr>
<td>I</td>
<td>Ra3</td>
<td>31.70</td>
<td>2.44</td>
<td>1.43</td>
<td>13.00</td>
<td>19.52</td>
<td>162.38</td>
<td>54.13</td>
</tr>
<tr>
<td>I</td>
<td>Ra7</td>
<td>33.75</td>
<td>2.11</td>
<td>1.26</td>
<td>16.00</td>
<td>18.09</td>
<td>186.60</td>
<td>0.62</td>
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<tr>
<td>I</td>
<td>REM2</td>
<td>6.74</td>
<td>4.83</td>
<td>1.57</td>
<td>0.38</td>
<td>4.00</td>
<td>2.00</td>
<td>5.24</td>
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<tr>
<td>II (weak)</td>
<td>Ra8</td>
<td>22.75</td>
<td>21.27</td>
<td>2.54</td>
<td>0.43</td>
<td>9.00</td>
<td>7.55</td>
<td>12.96</td>
</tr>
<tr>
<td>II (weak)</td>
<td>Ra9</td>
<td>23.37</td>
<td>0.87</td>
<td>0.15</td>
<td>25.00</td>
<td>11.41</td>
<td>184.89</td>
<td>61.63</td>
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<tr>
<td>II</td>
<td>Ra5</td>
<td>48.25</td>
<td>8.96</td>
<td>2.45</td>
<td>0.24</td>
<td>20.00</td>
<td>5.66</td>
<td>22.95</td>
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<td>II</td>
<td>REM1</td>
<td>17.71</td>
<td>12.73</td>
<td>1.75</td>
<td>0.69</td>
<td>9.33</td>
<td>3.21</td>
<td>14.96</td>
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<tr>
<td>II</td>
<td>REM3</td>
<td>38.59</td>
<td>15.61</td>
<td>1.45</td>
<td>0.35</td>
<td>26.00</td>
<td>4.36</td>
<td>19.06</td>
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<tr>
<td>IV</td>
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<td>17.42</td>
<td>0.87</td>
<td>0.15</td>
<td>25.00</td>
<td>16.37</td>
<td>11.41</td>
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<tr>
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<td>18.60</td>
<td>8.46</td>
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<td>0.28</td>
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<td>6.95</td>
<td>1.43</td>
<td>0.30</td>
<td>16.00</td>
<td>3.61</td>
<td>17.01</td>
</tr>
</tbody>
</table>

Values were taken from one to three 5-hour recording days. Normal group values are based on a single 5-hour baseline recording session for 2 non-lesioned animals that were used for comparison and 8 non-lesioned animals that participated in other studies.

Behavior

Figure 2 illustrates representative lesions that encompass the size and locations of those inducing the various behavioral categories. A relatively small unilateral lesion was sufficient to eliminate atonia determined polygraphically but not to allow overt behavior. This rat was still included in Group I. Bilateral lesions in either RPO or RPC led to behavioral release during REM-A. The behaviors ranged from increased muscle tone with exaggerated twitches and jerks (Group I, n=5, not including the rat with a unilateral lesion), to head rocking and head lifting (Group II, n=9), to extensive movements of the fore- and hind limbs (Group IV, n=3). Four of the nine rats exhibiting Group II behaviors were not recorded from and are not included in Table 1. The three rats with the greatest release during REM-A (Group IV) exhibited more extensive behaviors, including headlifting, treading limbs as if walking in place, full locomotion, and explosive leaps and jumps, which often terminated the episode. The Group II, and Group III categories were not found in these animals. The behaviors were only observed during electrographically identified REM.

However, we found that full Group IV behavior could not be expressed when the rat was connected to the recording cable, so we first checked for Group IV behavior with the rat moving freely. The character of the Group IV behavior was clearly different from that observed during wakefulness (Video 2).

In a subset of animals (n=7) we studied in detail the transitions into REM. Six of these rats had direct transitions into REM-A from wakefulness in 31 of 531 REM-A episodes. This phenomenon may not be related to behavioral release since the sleep-onset REM episodes were not observed in one Group IV animal.

Elaborate behaviors during REM-A continued for several days post-lesion. Normal REM episodes were never interspersed with REM-A episodes. When behavior disappeared during REM, atonia was again a feature of each episode.

Figure 2. Diagrams of Histological Sections Showing Sample Lesions Producing Groups I, II and IV Behaviors. Colors depict lesions in different rats. All were bilateral except the Group I rat lesion pictured in brown.
Histology

Lesions releasing elaborate behaviors during REM-A were located bilaterally in both RPO and RPC (Figure 2). Thus far, we have not been able to distinguish between the effects of lesions in RPC and RPO in their ability to produce behavior in REM-A. That is, damage in both areas appears to have the potential to release up to Group IV (full locomotion) behaviors. This may change as we continue to explore variations in lesion site and size.

DISCUSSION

The present results further demonstrate that REM-A can be produced in the rat. Behaviors released can be complex and are similar to those observed in cats (Jouvet and Delorme, 1965; Henley and Morrison, 1974; Hendricks et al., 1982) and previously briefly described in rats (Mouret et al., 1967; Mirmiran, 1983).

Comparison of Behavioral Release in Rats and Cats

We observed behaviors we would characterize as Group I, Group II, and Group IV, based on the categories applied to cats. We did not observe predatory attack (Group III) in rats. However, attack behaviors have been reported to occur (Mirmiran, 1983), and we may see them as we examine different lesion sites in the pons.

In the present group of animals, elaborate behaviors disappeared a week or so post-lesion. Although behavior in some cats in our previous studies was no longer evident within days or a few weeks after lesion placement, other cats continued to have REM-A episodes with behavior for months (Henley and Morrison, 1974; Hendricks et al., 1982). It remains to be seen if the phenomenon can last as long in rats. Also, cats that lost the ability to right and lift their heads, and even bodies, continued to have excessive truncal movements during REM much like those characterized as Group I. This was not observed in the rats when they recovered from elaborate behavioral release. Electrical stimulations of the region with lesions in the present study that were performed in decerebrate rats by Hajnik et al. (2000) revealed an apparent overlap of areas facilitating and inhibiting muscle tone. These regions are separated in cats. Thus, the effects we observed may have been more easily neutralized by damage to both inhibitory and facilitatory areas in the rat, leading to more rapid recovery of antonia than observed in cats.

The lengths of REM-A episodes in many of the rats were shorter than the average normal REM episode duration found for the control population, although it was not possible to study this statistically. While feedback from gross movements could have contributed to the shortening of their REM-A episodes, this is probably not the only reason. Of the two rats with the shortest episodes, one was from a group with weak movements and the other was from the group with the most elaborate movements. Sanford et al. (1994) found the same lack of association between degree of behavioral release and duration of episodes in cats. This suggests that at least some lesions are interfering with a more central REM-controlling mechanism. Trulson et al. (1981) demonstrated that normally silent serotonergic dorsal raphe neurons resumed varying degrees of activity once cats entered REM-A. The release of serotonergic activity may be sufficient to interfere with the maintenance of REM, and one site of this action is, likely, the central nucleus of the amygdala (Sanford et al., 1995).

The release of wake-like behaviors during REM-A raises the question of how the animal perceives the external environment during REM-A. Morrison (1983) has long held the view that brain activity during REM and waking share fundamental similarities. The virtually indistinguishable cellular activity during W and REM in much of the brain, particularly thalamocortical regions, has, more recently, led Llinás and Paré (Llinás and Paré, 1991; Paré and Llinás, 1995) to essentially the same conclusion. In their conception, W and REM "are fundamentally identical states, with the provision that the handling of sensory information is altered in REM" (Paré and Llinás, 1995). They note that sensory responsiveness, as indicated by evoked potentials, is more alike in W and REM than either is to NREM, though higher intensity stimuli are required for awakening from REM.

In fact, in cats, we demonstrated that external stimuli can elicit overt behavioral OR during REM-A without producing arousal (Morrison et al., 1995). Two cats capable of locomotion in REM-A turned their heads toward speakers producing tone stimuli of varying intensities. Two other cats, one capable of lifting its head and one with head lifting and spontaneous OR, but neither exhibiting locomotion, exhibited ear pinna rotation but no head turning in response to tones. Two other cats with more release of muscle tone, one capable of righting with its forequarters, did not exhibit OR to external stimuli in REM-A. All cats showed OR in W and none did in NREM. In the four cats that exhibited elicited behavioral OR in both W and REM-A, responses to tones of different intensities were of a similar quality and occurred on approximately the same number of trials in both states. All of the cats tested exhibited the acoustic startle response as measured by ear flicks and/or head and body twitches in response to tones, and our measures of OR (head OR and ear pinna rotation) were distinguishable from these responses, suggesting a different level of information processing. The development of the REM-A model in rats may allow a greater exploration of response capabilities in REM-Adue to the greater ease in producing larger numbers of rats with REM-A compared to cats.

REM-A as a Model of REM Behavior Disorder (RBD)

REM-A in animals may be viewed as an animal model of RBD in humans. Both are characterized by a release of behavior in REM (Schenck et al., 1996). RBD is characterized by "vigorous and frequently violent dream-enacting behaviors" and is most often observed in older men (Schenck et al., 1986; Mahowald and Schenck, 1994). RBD is associated with several neurological diseases and often occurs in conjunction with the onset of narcolepsy, neurodegenerative, cerebrovascular, and other neurologic disorders (Schenck and Mahowald, 1990; Mahowald and Schenck, 1994). The relationship to narcolepsy is interesting because we observed instances of polygraphically identified wake-onset REM-A episodes; however, at present, these observations are too premature to describe in a systematic fashion. As we continue to develop the REM-A model in rats and the number of animals exhibiting wake-onset REM-Apotentially increases, it may become possible to distinguish these animals on a neuroanatomical basis, if not by behavioral category.

Fifty-five percent of RBD is idiopathic with no identifiable neuropathology. This observation prompted Mahowald and Schenck (1994) to suggest that idiopathic RBD may result from subtle changes in the brain that compromise descending inhibition to the brainstem and that structural damage to the
pons, such as that produced by lesions producing REM-A in animals, may rarely be the cause of RBD in humans. However, RBD may be the first sign of multiple system atrophy that more fully manifests itself over time. RBD may herald Parkinson’s disease in older RBD patients, and a number of presumed Parkinson’s disease cases may eventually be diagnosed as multiple system atrophy (striatonigral degeneration subtype) (Schenck and Mahowald, 1990). RBD can precede olivopontocerebellar atrophy and striatonigral degeneration, both of which are almost always associated with pontine lesions (Schenck and Mahowald, 1992). Thus, a fuller understanding of pontine damage and behavioral release in REM-Ain animal models will almost certainly lead to insights into human RBD.

Conclusions

The present study confirms that REM-A can be created successfully in rats and extends two earlier preliminary reports (Mouret et al., 1967; Mirmiran, 1983) by demonstrating that the behaviors released can be elaborate and complex, depending upon lesion site. There is increasing recognition that exploration rostral to the pons will be necessary to unravel REM mechanisms completely (Morrison and Reiner, 1985; Morrison et al., 1999), which may be accomplished more conveniently in rats. For example, in the case of motor control, structures rostral to the pons likely contribute to the normal atonia of REM and to the behavior released during REM-A (Morrison, 1988; Rye, 1997; Zagrodska et al., 1998). These facts must be considered as work progresses on the problem of REM Behavior Disorder.

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REFERENCES