Mesopontine contribution to the expression of active ‘twitch’ sleep in decerebrate week-old rats

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Abstract

Myoclonic twitching is a ubiquitous feature of infant behavior that has been used as an index of active sleep. Although the active sleep of infants differs in some ways from the REM sleep of adults, their marked similarities have led many to view them them as homologous behavioral states. Recently, however, this view has been challenged. One avenue for resolving this issue entails examination of the neural substrates of active sleep. If the neural substrates of active sleep were found to be similar to those of REM sleep, then this would support the view that the two states are homologous. Therefore, in the present study, decerebrations were performed in the pons and midbrain to determine whether the mesopontine region is important for the expression of active sleep in infants, just as it is for the expression of REM sleep in adults. It was found that, in comparison to controls, caudal pontine decerebrations reduced myoclonic twitching by 76%, rostral pontine decerebrations reduced twitching by 40%, and midbrain transections had no significant effect on twitching. Moreover, analysis of the temporal organization of twitching indicated that pontine decerebrations predominantly affected high-frequency twitching while leaving unaffected the low-frequency twitching that is thought to be contributed by local spinal circuits at this age. These results indicate that the mesopontine region plays a central role in the expression of active sleep in infant rats.

Theme: Neural basis of behaviour
Topic: Biological rhythms and sleep
Keywords: Myoclonic twitching; Active sleep; REM sleep; Rat; Infant; Decerebration

1. Introduction

Spontaneous neural activity is a ubiquitous feature of developing nervous systems [7,18]. Within the neuromuscular system, spontaneous activity in the form of myoclonic twitching is readily observed in the fetuses and infants of many mammalian species, including rats [21,26], sheep [9], and humans [30]. In fetal and infant rats, myoclonic twitching is exhibited throughout the body, especially by the distal limbs and head [17,21]. Spinal transection experiments in perinatal rats have shown that this twitching is generated spontaneously by neural elements within the spinal cord and, as development proceeds, falls under the increasing influence of neural structures in the brain [2,29].

Phenomenologically, the myoclonic twitching of infant rats appears homologous with the twitching exhibited by adults during REM sleep [7]. But myoclonic twitching is but one of many components that characterize the adult state of REM sleep; these other components include rapid eye movements, muscle atonia, PGO waves, and an activated EEG. Thus, to use Corner’s [8] metaphor, REM sleep in adult rats is like a rope comprised of multiple strands, and as we trace back the steps from adult to infant, the rope unravels until we are left, at birth, with the single remaining strand of myoclonic twitching [3]. Accordingly, some researchers interested in the expression of sleep in infant rats have used myoclonic twitching as their primary index of active sleep [5,17,21,33]. Indeed, observations of...
infant rats at thermoneutrality and during cold exposure has revealed that the expression of myoclonic twitching is an extremely useful and reliable measure of behavioral state [5,33].

The above discussion highlights the centrality of myoclonic twitching for the study of active sleep. Nonetheless, it remains unclear whether active sleep is homologous to REM sleep. For example, one group of investigators has argued that active sleep in infant rats is an undifferentiated ‘protostate’ out of which both REM and slow-wave sleep eventually develop [11–14]. These studies, however, use the EEG as the primary index of sleep state and, most significantly, focus exclusively on infant rats 11 days of age and older (when traditional methods of EEG analysis become easier to apply to infants). This approach, although valid with regard to the study of the development of neocortical activity, provides little insight into the development of the more fundamental brainstem mechanisms that are known, at least in adults, to underlie the control and modulation of REM sleep [19,24,35].

If myoclonic twitching in infants during active sleep is homologous to that expressed in adults during REM sleep, then the neurological substrates of active and REM sleep should be similar. With regard to adults, the current prevailing view is that cholinergic neurons in the mesopontine tegmentum, especially the laterodorsal tegmental and pedunculopontine tegmental nuclei, play a critical role in the generation of REM sleep [19,24]. According to this perspective, efferents from these mesopontine nuclei are responsible for triggering the expression of the other components of REM sleep, including PGO waves, activated EEG, muscle atonia, and myoclonic twitching [35]. Moreover, for each REM component, brainstem nuclei have been identified that are necessary for that component’s expression. For example, normal expression of myoclonic twitching during REM sleep appears to depend on the nucleus pontis caudalis and the nucleus gigantocellularis and their descending fibers within the reticulospinal tract [35].

Although the neurological substrates of REM sleep in adults have been well characterized, the same cannot be said for active sleep in infants. Therefore, in the present series of experiments, we investigated the neurological substrates of active sleep in week-old rats using myoclonic twitching as an index of this behavioral state. In these experiments, the brainstem was transected at three levels and myoclonic twitching and awake behavior were measured. Consistent with earlier decerebration experiments in adults [20], it is demonstrated that decerebrations in the caudal pons significantly inhibit myoclonic twitching while decerebrations in the caudal midbrain have no effect. These experiments indicate that myoclonic twitching can be used effectively as an index of active sleep for investigations of the neurological substrates of sleep/wake behaviors in developing rats.

2. Materials and methods

2.1. Experiment 1

2.1.1. Subjects

In Experiment 1a, the subjects were twenty-four 7–8-day-old Sprague–Dawley rat pups (Rattus norvegicus) from 8 litters. All pups weighed 11.2–20.5 g at the time of testing. In Experiment 1b, the subjects were eight 7–8-day-old Sprague–Dawley rat pups from 4 litters. These pups weighed 14.2–19.5 g at the time of testing. All pups were born to mothers in the animal colony at the University of Iowa. Mothers and pups were maintained in a temperature-controlled room with a 12/12 h photoperiod cycle (lights on at 0600) and housed in standard laboratory cages (48×20×26 cm) in which food and water were available ad libitum. All litters were culled to 8 pups within 3 days after birth (day of birth=Day 0). All subjects were treated in accordance with the Guide for the Care and Use of Laboratory Animals, DHEW Publication No. (NIH) 86-23.

2.1.2. Surgery

Under ether anesthesia, an incision was made exposing the skull, and a 23 gauge needle was used to drill a hole through the skull approximately 3 mm caudal to lambda. For decerebrate pups, a blunted 25 gauge needle was inserted into the hole and dragged from side to side to transect the brainstem. Sham-decerebrate pups underwent the same procedure except that the needle was not inserted through the hole in the skull. In Experiment 1b, all 8 subjects received caudal pontine decerebrations.

2.1.3. Procedure

On the day of testing, pups were weighed and their sex was determined. Littermates were randomly assigned to one of three experimental groups: decerebrate, sham-decerebrate, and nonsurgical control. The order of testing was determined using a balanced design. Following surgery, pups recovered in a thermoneutral (34.0–35.0°C) and humidified (58–65%) incubator for 90 min. Nonsurgical controls were placed in the incubator for 90 min but did not undergo surgery. Following this 90-min period, all pups were intubated with an oral catheter and received a preload of commercial half-and-half; the volume of this preload in ml was equal to approximately 4% of body weight. The provision of a preload of milk controlled for potential nutritional deficits due to separation from the dam during the experiment [22].

After receiving the preload of milk, pups were placed on their backs on a felt surface and secured in this position using 3 elastic straps. A supine position provides the observer with optimal visual access of twitching in each limb as well as awake behaviors [2]. Pups were allowed to acclimate to this position for 15 min. A microcamera placed above the pup was then used to record limb
movements to S-VHS videotape. Videotaping continued for 15 min.

Because decerebration in infants can disinhibit brown adipose tissue (BAT) thermogenesis [4], interscapular temperature \( T_{is} \) was measured using a hand-held thermometer and a chromel-constantan thermocouple (Omega, Stamford, CT) accurate to within 0.1°C. \( T_{is} \) was measured by applying the tip of the thermocouple against the skin above the brown fat pad in the interscapular region until a stable reading was attained. In Experiment 1a, \( T_{is} \) was measured once when videotaping was complete.

In Experiment 1b, 8 additional pups received caudal pontine decerebrations and 4 of these pups were ganglionically blocked to prevent disinhibition of BAT thermogenesis. Specifically, after surgery, pups were placed in the incubator for 60 min, at which time they were injected with the ganglionic blocker, chlorisondamine hydrochloride (5 mg/kg), or saline; chlorisondamine has been used effectively in the past to block BAT thermogenesis [33]. The subjects were thirty 7–8-day-old Sprague–Dawley rat pups (\textit{Rattus norvegicus}) from 10 litters. Pups were housed and raised as in Experiment 1. All pups weighed 2.2.1. Subjects

The subjects were thirty 7–8-day-old Sprague–Dawley rat pups (\textit{Rattus norvegicus}) from 10 litters. Pups were weighted into anaesthesia and then were secured on their backs for 15 min. Finally, videotaping began and continued for 15 min. \( T_{is} \) was measured at the end of the test.

Temporal and event data were imported into Statview 5.0 for analysis. For each 15-min recording period for each limb, the number of twitches was determined. In addition, inter-twitch interval (ITI) was defined as the interval between successive twitches regardless of whether awake behaviors were interposed. ITI provides a measure of the temporal organization of twitching within a limb. Finally, to determine duration of time awake for each pup, the time series for all four limbs were combined into a single data file. Then, for each bout of awake behavior, the duration of time from the first awake behavior to the last awake behavior was determined. These durations were added together to provide total awake duration for the 15-min period.

Differences between groups were tested using one-way and two-way analyses of variance (ANOVA). The post hoc test was Fisher’s protected least significant difference (PLSD) and \( \alpha \) was set at \( P<0.05 \). Unless otherwise stated, there were no significant differences between sham and nonsurgical controls. Therefore, although statistical analyses were performed on all three groups (i.e., decerebrate, sham, and nonsurgical controls), figures only present data for the decerebrates and sham. All means are presented with their standard errors.

Finally, to assess intrarater reliability, the observer occasionally scored the same 15-min period twice. The 2 data files for the same limb were imported into a custom program written in Hypercard for the Macintosh that divided each 15-min period of behavioral data into 90 10-s bins and determined the number of twitches observed in each 10-s bin. Binned data were then imported into Statview and the data for the two passes were correlated. These correlations were typically greater than 0.90, as in previous studies from our laboratory [2,29].

2.2. Experiment 2

2.2.1. Subjects

The subjects were thirty 7–8-day-old Sprague–Dawley rat pups (\textit{Rattus norvegicus}) from 10 litters. Pups were housed and raised as in Experiment 1. All pups weighed 11.09–21.49 g at the time of testing.

2.2.2. Procedures and analyses

All procedures and analyses were identical to those used in Experiment 1a except, during surgery, the hole was drilled 1 mm caudal to lambda.
2.3. Experiment 3

2.3.1. Subjects

The subjects were twenty-four 7–8-day-old Sprague–Dawley rat pups (Rattus norvegicus) from 8 litters. Pups were housed and raised as in Experiment 1. All pups weighed 10.8–15.8 g at the time of testing.

2.3.2. Procedures and analyses

All procedures and analyses were identical to those used in Experiments 1a and 2. During surgery, the hole was drilled 1 mm caudal to lambda, as it was in Experiment 2. In this experiment, however, the needle used to transect the brain was angled forward in order to achieve decerebrations that were located more rostrally than those in Experiment 2.

3. Results

3.1. Experiment 1

Fig. 1A presents a sagittal section of a week-old rat brain and depicts the approximate medial locations of the decerebrations for the 8 decerebrate subjects in Experiment 1a. As shown in the figure, the decerebrations began dorsally in the inferior colliculus and ended ventrally caudal to the pontine nucleus. Histological examination of the decerebrate brains indicated that, for all 8 brains, the transections passed through the posterior pons, just caudal to the pontine nucleus.

Caudal pontine decerebrations resulted in substantial reductions in myoclonic twitching, with significant main effects of group, $F_{(2,84)} = 256.2, P < 0.0001$, and limb, $F_{(3,84)} = 29.2, P < 0.0001$, and a significant group × limb interaction, $F_{(6,84)} = 8.9, P < 0.0001$. As shown in Fig. 1B, rates of twitching were reduced in all four limbs. (Again, the data for the nonsurgical controls are omitted from the figure for clarity.) On average, twitching rates in the decerebrate pups were reduced 81% in the forelimbs and 68% in the hindlimbs. While the main effect of limb reported above was significant, it is an expected result because forelimbs typically twitch at higher rates than hindlimbs [2,29].

It is possible that the reduction in twitching in the decerebrate pups was due either to stimulation of awake behavior or to the induction of a comatose state. Neither of these possibilities, however, appears to be the case. Specifically, although the mean awake durations for the decerebrate pups (24.1 ± 9.0 s) was less than those for the sham and nonsurgical controls (95.8 ± 27.3 and 81.5 ± 25.6 s, respectively), these differences were not significant, $F_{(2,21)} = 2.9$. These and other behavioral observations of the decerebrate pups indicate that they were capable of coordinated motor activity, thus suggesting that the observed reduction in twitching did not occur secondarily to generalized deficits in motor behavior. Nor was there any indication of decerebrate rigidity that could also have interfered with the expression of myoclonic twitching.

Previously, it was found that pups that experienced mid thoracic spinal transection at 2 days of age and were tested at 5 days of age exhibited a 50% reduction in hindlimb twitching [2]. Similarly, as discussed above, caudal pontine decerebrations in week-old rats resulted in a 68% reduction in hindlimb twitching rates. The difference between these two results, if reliable, could indicate a contribution to hindlimb twitching from neural sources lying between the midthoracic spinal cord and caudal pons. Alternatively, because the two experiments were performed on pups of different ages, the difference could reflect the diminished contribution to twitching of local spinal circuits as descending influences from the brain develop [8].

Fig. 1C presents the frequency distributions of ITI for the right forelimb and right hindlimb (these data are representative of all four limbs). Two-factor ANOVA performed on each limb separately revealed significant main effects of group (right forelimb: $F_{(2,210)} = 251.6, P < 0.0001$; right hindlimb: $F_{(2,210)} = 171.3, P < 0.0001$) and ITI (right forelimb: $F_{(2,210)} = 270.3, P < 0.0001$; right hindlimb: $F_{(2,210)} = 96.0, P < 0.0001$), and a significant group × ITI interaction (right forelimb: $F_{(18,210)} = 57.6, P < 0.0001$; right hindlimb: $F_{(18,210)} = 19.1, P < 0.0001$). As shown in the figure, twitching was disproportionately reduced at the smaller ITIs.

As discussed previously, pontine decerebrations disinhibit BAT thermogenesis in infant rats [4] and this was also found here. Specifically, mean $T_{ir}$ measured at the end of the 15-min test was 40.4 ± 0.4°C for the decerebrate pups, 38.1 ± 0.1°C for the sham pups, and 38.0 ± 0.2°C for the nonsurgical pups. These differences were significant, $F_{(2,21)} = 24.3, P < 0.0001$. Therefore, one possible explanation for the depressed twitching exhibited by the decerebrate pups in Experiment 1a was that they were hyperthermic in comparison with the control pups.

To address this possibility, Experiment 1b was conducted as a follow-up in which 4 pairs of pups received caudal pontine decerebrations and one pup in each pair was ganglionically blocked in order to inhibit BAT thermogenesis. As expected, decerebrate pups injected with the ganglionic blocker, chlorisondamine, had a significantly lower mean value of $T_{ir}$ at the end of the experiment than did the decerebrate pups injected with saline (chlorisondamine: 37.6 ± 0.2°C; saline: 40.2 ± 0.6°C; $t_{6} = 3.8, P < 0.01$). Despite this inhibition of BAT thermogenesis, pups in the two groups exhibited rates of twitching that were statistically indistinguishable from each other and similar to the decerebrate pups in Experiment 1a (see Fig. 1B). Specifically, mean rates of twitching for both groups across all limbs ranged between 37 to 77 twitches per 15 min. Finally, histological examination of
the brains of these pups indicated that the brains were transected at a level similar to those in Experiment 1a.

3.2. Experiment 2

Fig. 2A presents a sagittal section of a week-old rat brain and depicts the approximate medial locations of the decerebrations for the 8 decerebrate subjects in Experiment 2. As shown in the figure, the decerebrations began dorsally in the superior colliculus and ended ventrally within the rostral pons and caudal hypothalamus.

Rostral pontine decerebrations resulted in substantial reductions in myoclonic twitching, with significant main effects of group, $F_{(2,108)}=28.5$, $P<0.0001$, and limb,
Fig. 2. The effect of rostral pontine decerebrations on myoclonic twitching in Experiment 2. (A) Schematic drawing illustrating the range of decerebrations. Abbreviations as in Fig. 1. (B) Mean number of twitches (±S.E.M.) in each of the four limbs for decerebrate (solid bars) and sham (open bars) subjects during the 15-min testing period. *P<0.0005 compared to shams. (C) Frequency distributions of mean intertwitch intervals (±S.E.M.) for the right forelimb and the right hindlimb of decerebrate (solid bars) and sham (open bars) subjects. *P<0.01 compared to shams. For clarity, data for nonsurgical controls are not shown, although they did not differ from the data for sham controls.

\( F_{(3,108)} = 14.8, P<0.0001. \) The group×limb interaction was not significant. As shown in Fig. 2B, rates of twitching were reduced in all four limbs. On average, twitching rates in the decerebrate pups were reduced 44% in the forelimbs and 37% in the hindlimbs.

To examine the possibility that the reduction in twitching in the decerebrate pups was due to increased awake behavior, durations of awake behavior were again analyzed. Mean awake durations for the decerebrate pups (170.9±72.3 s) was greater than those for the sham and nonsurgical controls (68.6±17.2 and 25.6±2.7 s, respectively), but again these differences were not significant,
$F_{(2,27)} = 3.0$. The high level of awake behavior for the decerebrates was due to two subjects; when these subjects are excluded from the analysis, mean awake duration decreases to 76.3±31.8 s, and the group differences still are not significant, $F_{(2,25)} = 2.1$.

Fig. 2C presents the frequency distributions of ITI for the right forelimb and right hindlimb (again, these data are representative of all four limbs). Two-factor ANOVA performed on each limb separately revealed significant main effects of group (right forelimb: $F_{(2,270)} = 25.0, P < 0.0001$; right hindlimb: $F_{(2,270)} = 22.3, P < 0.0001$) and ITI (right forelimb: $F_{(9,270)} = 90.6, P < 0.0001$; right hindlimb: $F_{(9,270)} = 58.2, P < 0.0001$), and a significant group×ITI interaction (right forelimb: $F_{(18,270)} = 6.2, P < 0.0001$; right hindlimb: $F_{(18,270)} = 2.0, P < 0.01$). As shown in the figure, twitching was disproportionately reduced at the smaller ITIs.

In Experiment 1, caudal pontine decerebrations led to the disinhibition of BAT thermogenesis. In contrast, and as expected from previous findings [4], values of $T_{is}$ measured at the end of the experiment were similar across groups (decerebrate: 38.2±0.3°C; sham: 37.5±0.2°C; nonsurgical: 37.6±0.2°C). These differences were not significant, $F_{(2,26)} = 2.2$.

### 3.3. Experiment 3

Fig. 3A presents a sagittal section of a week-old rat brain and depicts the approximate medial locations of the decerebrations for the 8 decerebrate subjects in Experiment 3. As shown in the figure, the decerebrations began dorsally at the superior colliculus and ended ventrally within the caudal hypothalamus. Comparison with the decerebrations in Experiment 2 (see Fig. 2A) indicates some overlap, the primary difference being the angling of the cuts in Experiment 3 to achieve greater sparing of the anterior pons and caudal midbrain; this sparing was most evident upon histological examination of the lateral brain sections of the decerebrate subjects in Experiment 3.

Caudal midbrain decerebrations did not reduce myoclonic twitching. Specifically, although the main effect of limb was significant, $F_{(3,84)} = 22.5, P < 0.0001$, neither the main effect of group, $F_{(2,84)} = 2.0$, nor the group×limb interaction, $F_{(6,84)} = 0.5$, was significant. Furthermore, as in Experiments 1a and 2, there was no effect of group on the duration of awake behavior, $F_{(2,21)} = 1.0$, with mean awake durations of 17.8±5.0 s, 45.9±12.9 s, and 63.5±37.0 s for the decerebrate, sham, and nonsurgical pups, respectively.

Fig. 3C presents the frequency distributions of ITI for the right forelimb and right hindlimb. A two-factor ANOVA was performed for each limb separately. The main effects of group were not significant (right forelimb: $F_{(2,210)} = 1.7$; right hindlimb: $F_{(2,210)} = 1.3$), the main effects of ITI were significant (right forelimb: $F_{(9,210)} = 124.4, P < 0.0001$; right hindlimb: $F_{(9,210)} = 71.8, P < 0.0001$), and the group×ITI interaction was not significant (right forelimb: $F_{(18,210)} = 0.9$; right hindlimb: $F_{(18,210)} = 0.9$).

Mean $T_{is}$ measured at the end of the 15-min test was 37.4±0.2°C for the decerebrate pups, 38.1±0.1°C for the sham pups, and 38.1±0.1°C for the nonsurgical pups. These differences were significant, $F_{(2,21)} = 7.6, P<0.01$, indicating that decerebrate pups were mildly hypothermic.

### 4. Discussion

This series of experiments sought to identify the gross location of the neural substrates of active sleep in week-old rats. Whereas caudal pontine (Experiment 1a) and rostral pontine (Experiment 2) decerebrations led to reductions in twitching rates of approximately 76% and 40%, respectively, caudal midbrain decerebrations (Experiment 3) did not significantly reduce rates of twitching. All together, these findings suggest that mesopontine structures are sufficient for the expression of active sleep in infant rats, as is known to be the case in adults [19,24].

A reduction in twitching, such as that seen in Experiments 1a and 2, could arise for a variety of reasons. For example, if the decerebrations evoked coordinated awake behaviors, induced a comatose state, or produced decerebrate rigidity in the infants, then rates of myoclonic twitching would be reduced secondarily. None of these explanations, however, fits the data. First, the decerebrate subjects in Experiments 1a and 2 were capable of exhibiting coordinated motor patterns indicative of awake behavior, including stepping and stretching. Second, mean durations of awake behavior never differed between decerebrates, shams, and nonsurgical controls. Finally, observations of decerebrate subjects before and after the sleep tests indicated that they exhibited normal responses to handling by the experimenter.

It has been shown previously that decerebrations performed at certain levels of the neuraxis disinhibit BAT thermogenesis in newborn and infant rats [4]. Indeed, consistent with this earlier study, the decerebrate infants in Experiment 1a exhibited pronounced increases in intercapular temperature, indicative of increased BAT thermogenesis. To ensure that the reduced rates of twitching found in Experiment 1a were unconfounded by thermal effects, Experiment 1b was performed as a follow-up. In this experiment, caudal pontine decerebrations were performed as in Experiment 1a and half of the subjects were ganglionically blocked to suppress BAT thermogenesis. Suppression of BAT thermogenesis, however, had no effect on rates of twitching, indicating that the results of Experiment 1a were not due secondarily to hyperthermia.

In addition to rates of twitching, the temporal patterning of twitching was examined in these experiments. To do this, inter-twitch intervals were calculated for each limb and frequency distributions of ITIs were analyzed. These analyses indicated that the reduced rates of twitching found
in the decerebrate pups in Experiments 1a and 2 were largely isolated to the shortest ITIs, thus indicating that high-frequency twitching was primarily affected.

Log-survivor plots, such as those in Fig. 4, are also helpful for analyses of the temporal distribution of twitching [10]. The intervals between randomly-occurring events form a Poisson distribution that, on a log-survivor plot, fall along a straight line with a slope that is proportional to the rate at which events occur. The data in Fig. 4 are from the combined right forelimb ITIs of all decerebrate and sham pups in Experiments 1a, 2, and 3 (once again, data from nonsurgical pups are excluded for clarity, and they did not...
differ from those for sham pups). For the sham pups in all three experiments, as well as for the decerebrate pups in Experiment 3, the log-survivor plots in Fig. 4 indicate the presence of two processes. The first process is characterized by an excess of short ITIs (≤5 s), and the second process is characterized by longer ITIs that are distributed randomly with respect to time (as indicated by the data falling along a straight line for ITIs greater than 5 s). In contrast, the plot for the decerebrate pups in Experiment 1a is characterized by a single process in which all ITIs are distributed randomly with respect to time. Based on earlier work examining the contributions of local spinal circuits to myoclonic twitching [2], it appears that the twitching exhibited in Experiment 1a by the decerebrate pups resulted primarily from the spontaneous activity of spinal motoneurons (including perhaps some contribution from neurons in the nucleus gigantocellularis; see below) while the majority of twitching exhibited by the control pups resulted from supraspinal sources of activation.

Evidence from several sources suggests that the relative contribution of supraspinal sources to myoclonic twitching increases during development. Specifically, high cervical transection in fetal rats on the 20th day of gestation had no effect on rates of spontaneous limb activity [29] and midthoracic transection in 2-day-old rats tested at 5 days of age reduced myoclonic twitching in the hindlimbs by 50% [2]. In contrast, as shown in Experiment 1a, caudal pontine decerebration in week-old rats reduced myoclonic twitching in the hindlimbs by 68%. These results suggest a developmental phenomenon described previously by Corner (1985) in which local spinal control of motor behavior gives way to more rostral control as development proceeds. Additional work is needed, however, to provide a more complete description of the changing contributions of spinal and supraspinal sources to myoclonic twitching in perinatal rats.

There is a growing consensus that nuclei within the mesopontine tegmentum are primarily responsible for the generation of REM sleep [19,24]. Efferents from these nuclei are thought to activate an array of brainstem nuclei that are directly responsible for the expression of the individual components of REM [35]. Particularly relevant here is that myoclonic twitching is thought to be mediated primarily by the reticulospinal system, but not the pyramidal, rubrospinal, or vestibulospinal systems [15,16,23,35]. Specifically, experiments employing single-unit recordings in the nucleus gigantocellularis and the nucleus pontis caudalis have implicated these nuclei as sources of myclonic twitching in the adult [15,16,35]. With regard to the present study, it is apparent that the transections in Experiment 1a at least partially disrupted these reticulospinal outputs but that the more rostral transections in Experiments 2 and 3 did not.

At this time we can only speculate as to the specific neural structure or structures that contribute to the expression of active sleep and myoclonic twitching in infants.
Rat brain atlases for fetuses [1] and adults [27] differ significantly with respect to the differentiation and relative positions of nuclei, thus creating obvious difficulties when examining brains from the early postpartum period. In some cases (e.g., pontine nucleus) comparison between infants and adults is easy, but for more diffuse nuclei (e.g., nucleus pontis oralis) difficulties can arise. For example, in their atlas of the fetal brain, Altman and Bayer [1] do not identify individual pontine reticular nuclei and go no further than to identify the anterior and posterior pons. With these caveats in mind, the present results suggest that the mesopontine region is sufficient for the normal expression of active sleep in infants, as it is for REM sleep in adults. This suggestion follows from the following observations: First, rates of myoclonic twitching were normal in pups with caudal midbrain decerebrations but were severely depressed in pups with caudal pontine decerebrations; and second, focussing on the relatively small differences between the decerebrations in Experiments 2 and 3, increased sparing of neural tissue in the mesopontine region was sufficient to restore normal rates of twitching.

Although some investigators currently view active sleep as an undifferentiated state from which both REM sleep and slow-wave sleep develop [12], an alternative perspective holds that REM sleep is comprised of multiple components that coalesce during development [3]. Moreover, according to this latter perspective, each component may exhibit unique developmental profiles and functional characteristics. Therefore, we have focussed here on myoclonic twitching as a single yet dominant component of active sleep in the infant rat. Nonetheless, because of this exclusive focus, the present results should be viewed with caution. Similar examinations of other components, including their temporal coalescence during development, will be necessary to answer the larger questions regarding the development of sleep in infants and its relation to sleep in adults.

Spontaneous neural activity contributes to the development and organization of the nervous system [31]. For example, in chick embryos, chronic blockade of neuromuscular activity prevents programmed cell death [28] and, in neonatal rats, loss of normal activity at the neuromuscular junction leads to the retention of polynuclear innervation at motor endplates [6,34]. In addition, blockade of spontaneous activity of retinal ganglion cells in fetal cats disrupts the normal formation of ocular dominance columns [32] and the loss of normal visual input to one eye alters the neural organization of the visual input to striate cortex [36]. Together, these examples illustrate activity-dependent processes that shape the developing nervous system. In contrast, while it has been hypothesized that myoclonic twitching and other features of active sleep play similar developmental roles in nervous system organization [3,25,30], there have been few direct tests of these hypotheses. Therefore, it is hoped that the present study, by contributing to the identification of the neural substrates of active sleep and myoclonic twitching, will open new experimental avenues for exploring the functional significance of myoclonic twitching and its coalescence with other sleep-related behaviors during ontogeny.

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