Clomipramine Suppresses Postnatal REM Sleep Without Increasing Wakefulness: Implications for the Production of Depressive Behaviors

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Abstract: Clomipramine (CLI), a REM sleep suppressant, alleviates symptoms of depression in adults but produces depressive behaviors if applied neonatally. Both effects of CLI as applied to adults and to neonates have been interpreted as consequences of its involvement in REM sleep deprivation. However, the paradox of these conflicting effects remains to be understood. The current study attempts to find the possible answer by studying the effects of CLI on postnatal sleep. Eight postnatal rats were evaluated polysomnographically for nine days. Four rats were treated with CLI, 40 mg/kg/day for six days, and four rats were treated with equivolume saline during the same period. The results showed that 1) CLI treatment did not reduce the time of phasic muscle activity which appears during slow wave EEG as it did during REM sleep; 2) during treatment, rats treated with CLI had 44.66%-68.62% REM sleep reduction, varied according to age; 3) REM sleep reduction during treatment was generally compensated by non-REM sleep, so that total sleep (and wakefulness) was comparable to that experienced by rats treated with saline; 4) an obvious REM sleep rebound was observed after drug withdrawal at the age of P19. These results suggest that 1) the stage that shows phasic muscle activity simultaneously with a high amplitude EEG is not REM sleep and is likely to be independent from non-REM sleep in terms of the percentile change; 2) REM sleep reduction without a corresponding increase in wakefulness in postnatal rats is likely the mediator of postnatal RSD in the production of adult depression; and 3) the neuronal bases responsible for REM rebound function by the end of the postnatal third week.

Key words: Postnatal rats; soft head-plug; clomipramine; postnatal sleep; REM sleep; REM sleep deprivation; active sleep; depression; muscle twitches

INTRODUCTION

SERIAL STUDIES PERFORMED BY VOGEL AND COLLEAGUES AND OTHER LABORATORIES HAVE ADVANCED VOGEL’S HYPOTHESIS THAT NEONATAL REM SLEEP DEPRIVATION (RSD) produces adult depression and adult RSD alleviates depression. However, the controversial paradox of this difference in effects remains to be examined. A reasonable consideration might be that some difference exists between the effects of RSD on the sleep variables of neonates and those of adults.

Studies of sleep ontogeny demonstrate that the sleep patterns of mammals and humans in their very early lives are characterized by a very high percentage of REM sleep and very frequent phasic muscle activities/twitches (PMA). “Active sleep” is the term that has been used to describe these behavioral phenomena. However, active sleep may not be identical to adult REM sleep. Jouvet-Mounier and colleagues observed that active sleep actually includes two different states. One state involves rapid activity as recorded by cortical EEG, consisting of regular waves, theta rhythms, REM, low muscle tone, and PMA. This is a state that seemingly corresponds to adult REM sleep, or paradoxical sleep. Additionally, they also observed another state, which was behaviorally scored as active sleep but appears different from REM sleep in polysomnographic recording. This state consists of PMA activity, low muscle tone, high amplitude EEG, and a lack of eye movement. They called this phenomenon “half-activated PS” and “SWS (slow-wave sleep) with muscle twitches.” Such a state or phenomena has been confirmed by other laboratories, including ours, and was included in quantitative calculations of SWS in Jouvet-Mounier and colleagues’ study and in McGinty and colleagues’ study of kittens. Frank and Heller interpret active sleep as an undifferentiated state and postulate that both adult REM sleep and SWS are derived therefrom.

Interested in the function of neonatal REM sleep, Mirmiran and colleagues first studied the adult behavioral effects of neonatal RSD by administering clomipramine (CLI), an antidepressant and a powerful REM sleep suppressant. They found multiple behavioral abnormalities, including sexual behavior deficits in rats neonatally treated with CLI (CLI rat). Vogel and colleagues systemically studied the behavioral abnormalities of CLI rats. They found that CLI rats exhibited decreased sexual activity, decreased aggression as measured by shock-induced fighting, increased locomotion, and decreased pleasure seeking behaviors as measured by intracranial self-stimulation. CLI rats also showed abnormalities in sleep variables, including decreased REM latency, increased REM sleep, and an increased sleep onset REM period. Many behavioral abnormalities found in CLI rats have been replicated by other laboratories. CLI rats also manifested other depressive signs, such as increased alcohol consumption and increased immobility in a forced swimming test. Vogel interpreted these findings as a set of behavioral abnormalities seen in human endogenous depression and considered the CLI rat an animal model of human endogenous depression, now called major depressive disorder with melancholic fea-
tasures.1,18 This hypothesis is further supported by findings that imipramine used as an antidepressant in humans also alleviates depressive behaviors in CLI rats37 and that CLI rats also demonstrate serotonergic and hypothalamic pituitary axis abnormalities comparable to those observed in human endogenous depression.28-31 The major paradoxical question, however, still remains: why does CLI alleviate depression when administered to adults and causes depression when administered to neonates?

More data reveals that, in rats, the aforementioned behavioral abnormalities can be produced not only by neonatal administration of CLI, but also by other REM suppressants, such as desipramine, zimeldine, nomifensin, Lu 10-134-C9,32-34 as well as by instrumental means.35 The features of sleep variables during the developmental period are similar to those observed in endogenous depression.7 Most of the sleep variables mature between postnatal weeks two and four.7,36 Coincidentally, the CLI treatment window during which adult sexual behavior deficits may be produced is no later than postnatal weeks two and three.37 These findings support the hypothesis that RSD is the common path through which neonatally administered CLI produces adult depressive behavior. In addition, non-drug RSD in humans has produced antidepressive effects and indicates that RSD is important in behavior regulation.38 The summary of these findings strongly suggests that the effects of CLI on sleep variables might be significantly different in neonates and adults and that the study of the effects of CLI on neonatal sleep might provide important information for answering the paradox of how CLI alleviates depression in adults and produces depression in neonates.

Since the effects of CLI on adult sleep have been reported previously, the current study will focus on the effects of CLI on neonatal sleep.

METHODS

Animals

Postnatal Long-Evans rat pups were used in this experiment. All procedures were approved by the Institutional Animal Care and Use Committee of the Emory University School of Medicine.

Four pairs of male rat pups born from three different mothers were obtained from a commercial provider (Harland/Sprague-Dawleys, WI) and used in this experiment. All paired rat pups were from the same litter. Rat pups remained with their mothers until the day before electrode implantation and were singly housed post surgery in the recording chambers for recovery and adaptation to recording conditions. The temperature of the recording chambers was maintained at 27—28°C by a temperature controller that operated infrared lights to supply heat.39,40 Fresh formula (Enfamil, generally used for human infants) was supplied in a plastic bowl and was refreshed every eight hours. The same formula was also fed to the rats by feeding syringes manually every four hours in the first four days of the experiment. Thereafter, the feeding times were gradually reduced to zero. The study was conducted under a 12/12 hour light/dark cycle.

Design

Rat pups underwent electrode implantation prior to the recording and treatment period, which began on the postnatal thirteenth day (P13), and were divided into experimental and control groups. Four rat pups were in each group. The experimental group was treated with CLI, 20 mg/kg, sc, twice daily (40 mg/kg/day), and the control group (to control for maternal separation and injection) was treated with equivalent saline during the same time period. Polysomnographic recordings were conducted for nine days, which included six days of treatment and three days of recovery. Since REM sleep decreases as postnatal age increases, the present study sought to determine the effects of RSD by comparing the REM sleep post-RSD of CLI rats with the REM sleep of saline-treated, age-matched control rats.

Electrode Implantation and Polysomnographic Recording

The soft head-plug method was used for electrode implantation and continuous polysomnographic recordings.39,41 The EEG electrode used was a 20 cm length of thin but strong Teflon-coated wire. Under Metofane anesthesia, the wire was led by a suturing needle through the neonate’s soft skull to the epidural space. Then, with a U-turn, the wire was exited through the skull. In order to make electrical contact with tissue, 2 cm of the Teflon coating was removed from the end of the wire that contacted the skull. Another micro-wire was implanted in the same way 2-3 mm caudal to the first location to allow for bipolar recording of the EEG. The same type of wire was implanted in the nuchal for EMG recording. All wires exited the skin through a soft guide tube connected to a small suspending connector. The skin was closed with a silk suture carefully, and the guide tube containing wires was carefully secured to the skin. Physiological signals from the soft head-plug were sent to a polygraph via a multi-channel commutator and then through a data processor (DAP 3000a/212, Microstar Lab) to a computer for storage and offline scoring of sleep/wake states. Dap View for Windows was used as a data collection program (Microstar Lab). Continuous (24 hrs/day) paper and computer polysomnographic EEG and EMG recordings of the experimental rats and control rats were made throughout the RSD (six days) and post-RSD (three days) periods.

Sleep Scoring

Polysomnographic data was scored by computer automatically in 30-second epochs. Briefly, the program calculated mean EEG amplitude and mean EMG amplitude in each 30-second epoch and compared these amplitudes to reference EEG and EMG amplitudes of each sleep/wake state. States with low amplitude EEG and low amplitude EMG were scored as REM sleep; states with high amplitude EEG and low amplitude EMG were scored as non-REM sleep (NREM). Epochs in which the majority of higher amplitude EEG contained phasic muscle twitches were also scored as NREM sleep and named PMA-NS. The epoch of PMA-NS was counted manually for four days (D1, D2, D4, and D5). Epochs characterized by high amplitude EMG and low amplitude EEG were scored as waking states.7 All results scored by computer were carefully confirmed visually.

Data Analysis and Statistics

Data was presented in 24-hour sections matched exactly for age between experimental rats and control rats. In order to eval-
uate the relationship between PMA-NS and REM and NREM sleep, changes of PMA-NS during six days treatment were calculated as the percentage of PMA-NS divided by NREM sleep and by REM sleep in addition to the calculation of daily percentage. The lack of a significant difference in PMA-NS/REM sleep between the two groups during treatment indicates that the total time of PMA-NS changes proportionally to the changes of REM sleep and that PMA-NS derives from a similar source as REM sleep.

Statistical assessment of experimental (CLI treated) and control (saline treated) differences in all sleep-wake variables was conducted by two-way (treatment and day) repeated (rat) measure ANOVA and post-hoc pairwise comparisons by the Student-Newman-Keuls method. Differences that were reported yet that were not significant with a relatively high value of factor F in the assessment of ANOVA were further assessed by t-test.

RESULTS

General Conditions of Rats During Experiment

On the day following surgery, treatment and polysomnographic recording began for all rats. The first day of treatment was designated deprivation day 1 (D1), and the last day of treatment was designated deprivation day 6 (D6). Thereafter, three days of recovery (drug withdrawal) designated R1 to R3, were also polysomnographically recorded. As is usually observed, all rats lost some body weight after surgery; behaviorally, however, they were healthy.

PMA-NS

The PMA appeared in EMG recordings as a single spike or multi-wave bursts. On most occasions, the single spike represents a local (ear, foot, mouth, and the like) muscle movement. A single spike of very high amplitude represents a quick jump (behavior was observed and immediately matched in paper recording). However, gross locomotion or a slower jump usually shows a burst of PMA. The overall mean PMA-NS time in the four counted days was 9.56±1.70% (10.33%, 9.85%, 8.71% and 9.34 on D1, D2, D4, and D5 respectively) in CLI rats and 10.54±2.85% (8.20%, 9.55%, 14.3% and 11.4% on D1, D2, D4 and D5 respectively) in saline-treated control rats. Figure 1 shows the PMA-NS of CLI and control rats represented as a percentage of NREM sleep. The proportion of PMA-NS/NREM sleep of the four presented days (21.76±4.58%) in CLI rats was significantly lower than that (31.88±7.51%) observed in control rats (treatment, F=16.3 and p=0.0068). The differences in daily comparisons between treatment groups were significant only on D4 (p<0.05). Further assessment by t-test indicates that the differences were significant on both D2 and D4 (p=0.017 in D2 and p=0.087 in D4).

In contrast, a significant, large, and consistent increment of the proportion of PMA-NS/REM sleep was found (Figure 2) in CLI rats during CLI treatment. The overall difference of this ratio between CLI and control rats was significant (treatment, F=363.08 and p<0.0001). The assessment of daily differences also displayed significance (group X day, F=5.51, p=0.01). Further assessment by t-test indicates that the differences were significant on both D2 and D4 (p=0.417 in D2 and p=0.087 in D4).

REM Sleep

Figure 3 shows the daily REM sleep percentage (mean±std) of both CLI and control rats. Saline-treated control rats had an average of 33.1% (±2.01%) REM sleep during treatment day D1 (P13) that gradually decreased to 19.47% (±2.83%) by D6 (P18) and 15.06% (±0.65%) by R3 (P21). In contrast, the CLI rats experienced only 13.29% (±1.98%) REM sleep on treatment day.
D1, a 60% reduction as compared to the control rats. Compared to control rats during the same period, mean daily REM sleep reduction during the six treatment days was 55.8±7.3% (Fig. 3). Statistics show 1) that the overall and daily differences of REM sleep between CLI and control rats were significant (treatment was F=256.4; p<0.0001 and daily comparison was p<0.05 in all six treatment days from D1 to D6) by repeated measures ANOVA; 2) that all p-values in daily comparison by t-test were less than or equal to 0.0001 except on treatment day D5, on which the p-value was 0.0019. The percentage of REM sleep and total sleep on the first and the last treatment day were 23.44±2.8% and 16.41±0.85% in CLI rats, and 52.52±3.19% and 33.73±4.93% in control rats.

On the first day of drug withdrawal, CLI rats had an estimable REM rebound. Compared to control rats, the REM sleep of CLI rats increased 57.2% on R1 and 34.7% on R2. The t-test showed that p<0.0001 and 0.03 respectively. REM sleep upsurge diminished rapidly. By the third day of recovery, the REM sleep of CLI rats was 15.8%, only a little bit higher than that of saline rats (15.1%), and statistics show that the difference was not significant (p=0.45).

**Non REM Sleep**

Figure 4 shows the daily average NREM sleep percentage of both groups. NREM sleep of CLI rats also underwent dramatic changes during the RSD and recovery period. The mean NREM sleep of CLI rats reached to 42.02% on the first day and to 50.75% on the last day of treatment (P19). Compared to saline rats, the NREM sleep of CLI rats increased 45.1% and 23.3% on the first day and the last day of RSD. The NREM sleep of CLI rats was even higher on the second day of RSD than on the first. The smallest increase was 17.1% (D5). The mean increment of NREM sleep was 39.1%. Statistics show 1) that the overall and most of the daily differences of NREM sleep between CLI and control rats were significant (treatment was F=82.28; p=0.0001 and daily comparison was p<0.05 in all six treatment days except D5, which was p=0.05, p=6, q=4.0263) by repeated measures ANOVA; 2) that all daily differences between CLI and saline rats were further assessed by t-test and the results were p<0.005, 0.003, 0.0008, 0.0007, 0.0177, and 0.01, respectively, from treatment day D1 to D6.

In contrast to the treatment period, the NREM sleep of CLI rats sharply dropped on the first day of drug withdrawal; graphically, the drop describes a nice seesaw form. The mean NREM sleep of CLI rats on recovery day R1 was only 32.8%, which was 16.3% lower than the mean NREM sleep of control rats on the same day (NREM sleep=39.2%) and 30.5% lower than the mean NREM sleep (47.2%) the same group had exhibited the day before (D6). Comparison of the NREM sleep difference between CLI rats and control rats on recovery day R1 shows a statistically significant difference (p<0.04), and the comparison of NREM sleep differences in CLI rats between treatment day D6 and recovery day R1 also shows statistical significance (p<0.05). Like REM sleep, NREM sleep also underwent a rapid adjustment after treatment withdrawal. However, it adjusted from a different direction.

**Relationship of REM Sleep to NREM Sleep During/After RSD**

Figure 5 shows directionally countervailing curves of REM sleep and NREM sleep during RSD and the recovery period in CLI rats. While REM sleep decreased during RSD, NREM sleep increased. Whereas REM sleep increased post-RSD, NREM sleep decreased obviously. The inverse correlation was strongly depicted.

**Total Sleep and Waking Changes**

Table 1 shows daily total sleep as a percentage of time (24 hours). The mean total sleep of control rats was initially at a slightly higher level (from about 63% to about 54% on the last day recorded). The overall mean percentage of total sleep was 57.1±2.97% in CLI rats and 61.6±1.56% in control rats. The total
sleep of the CLI group, however, did not exhibit the dramatic alteration evinced by compensatory changes of NREM sleep to REM sleep loss over the nine days of daily recording. There were, of course, some significant differences in the daily percentage of total sleep between the CLI and control groups as indicated by Table 1. Two-way repeated measures ANOVA showed that the overall difference between CLI and saline-treated rats was significant (treatment, F=14.7 and p=0.0086) but none of the p-values was less than 0.05 in all daily differences. Further assessment by t-test showed significance in the differences of D2, D3, and D4, but caution in interpretation of the significance is necessary because the powers in these days were below required values (see text).

### Table 1—Daily percentage of total sleep (M: mean; STD: standard derivation).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day</th>
<th>AVG</th>
<th>STD</th>
<th>AVG</th>
<th>STD</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AVG</td>
<td>CLI</td>
<td>Control</td>
<td></td>
<td></td>
</tr>
<tr>
<td>D1</td>
<td>56.70%</td>
<td>5.82%</td>
<td>63.01%</td>
<td>1.10%</td>
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<tr>
<td>D2</td>
<td>55.56%*</td>
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<td>2.72%</td>
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<tr>
<td>D3</td>
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<td>1.61%</td>
<td>63.82%</td>
<td>2.74%</td>
<td></td>
</tr>
<tr>
<td>D4</td>
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<td>61.35%</td>
<td>0.42%</td>
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<tr>
<td>D5</td>
<td>58.27%</td>
<td>2.85%</td>
<td>60.98%</td>
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</tr>
<tr>
<td>D6</td>
<td>56.44%</td>
<td>3.26%</td>
<td>57.72%</td>
<td>0.36%</td>
<td></td>
</tr>
<tr>
<td>R1</td>
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<td>1.11%</td>
<td>56.75%</td>
<td>3.41%</td>
<td></td>
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<td>3.85%</td>
<td>55.08%</td>
<td>3.75%</td>
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Our first finding is that the period of PMA-NS is more strongly resembles NREM sleep rather than REM sleep. This is indicated by the fact that 1) the mean of overall PMA-NS was not significantly reduced by CLI as REM sleep was and 2) the proportion of PMA-NS/REM sleep was significantly increased, which resulted primarily from the decrease of REM sleep. PMA-NS was found in our present and previous studies. In terms of polysomnographic recording, the state of PMA-NS, or the “half-activated state,” as it is called by Jouvet-Mounier, looks like NREM sleep because of the slower frequency and the higher amplitude of EEG, as well as the reduction of muscle tone. Behaviorally, however, such a state could be scored as active sleep because that the rats could be seen as “sleep” and “active” by behavioral observation. In the current study, Figure 1 displays the proportion of PMA-NS divided by the NREM sleep amount and the reduction of this proportion in CLI rats when compared with the control rats. This reduction did not primarily result from the reduction of PMA-NS (9.56±1.70% in CLI and 10.54±2.85% in control rats) but from the increase of NREM sleep (Figure 4). This finding indicates that PMA-NS is not affected by RSD and is likely independent of the changes to NREM sleep (which did not increase as NREM sleep increased). Figure 2 displays the same PMA-NS data divided by the amount of REM. This figure demonstrates a large and significant increase in the PMA-NS pro-
portion in CLI rats when compared to that of control rats during RSD by CLI. This increase did not result from the increase of PMS-NS itself but from the decrease of REM sleep (Figure 3). This indicates that CLI suppressed REM sleep without suppressing PMA-NS and that PMA-NS is not mechanized by the same neuronal basis as REM sleep. In previous studies, we found that the percentage of PMA-NS gradually decreased as NREM sleep increased between two and four weeks of age. PMA was also found in NREM sleep in adult rats and adult mice in our data (unpublished data). Findings of the current study indicate that the time of the PMA-NS state depends neither upon changes of REM sleep nor changes of NREM sleep. This fact further indicates that the EEG is more reliable than muscle twitches in terms of scoring sleep. These findings also imply that phasic muscle twitches are not derived from the same neuronal bases that controls tonic muscle tone, and that phasic muscle activity is not a reliable variable for scoring REM sleep.40 The lack of descending inhibition in the developmental period might be the cause of spreading muscle twitches.

Second, our most significant finding is that CLI did not produce a comparable waking increase while producing a dramatic REM sleep reduction, (i.e., the REM sleep reduction) was counterbalanced primarily by compensatory NREM sleep rather than wakefulness. This is significantly different from findings of the effects of CLI on sleep in adults.

First, CLI produced a dramatic REM sleep reduction in postnatal rats. As displayed in Figure 3, our study demonstrated a dramatic REM sleep reduction in CLI-treated postnatal rats. Compared with saline-treated control rats, CLI-treated postnatal rats showed solid REM sleep reduction in a daily average range from 44.66%–68.62% (varied according to age) and an overall mean REM sleep reduction of 55.8±7.3% (Figure 3). Because the saline-treated control rats were exactly matched to experimental rats in terms of age, litter mat, maternal separation, surgical procedure, ambient temperature, light-dark cycle, feeding procedure, recording method, and scoring parameters, the result of REM sleep reduction is solely the effect of CLI itself. Except for slight differences in the percentage of REM sleep reduction, these results are consistent with those of previous studies.2,3 The slight differences in REM sleep reduction between Mirmiran and colleagues’ study and ours might result from differences in recording and scoring methods. This finding, that CLI treatment reduces REM sleep in postnatal rats, indicates that postnatal treatment with CLI is capable of inducing adult depression-like behaviors via the reduction of postnatal REM sleep. A few previous studies reported that no REM sleep reduction was found during treatment with several REM sleep suppressants in postnatal rats.12,13,43,44 Three possible factors—dosages, recording method and scoring standard, and environmental temperature—might account for the failure to observe REM sleep reduction. In terms of drug amount per kg of body weight, it is generally understood that much higher doses of identical drug are needed in immature animals and humans than mature ones. Long-term polysomnographic recording should accordingly be more accurate.7,36,41 Our data demonstrated that the neutral ambient temperature for postnatal rats at P13 is 27%–28°C rather that 32°C,40 and the actual rectal temperature of neonatal rats is 34.67±0.32°C (at P13, n=10) while they stay with their mother rather equal to or higher than 36°C, as in adult rats.

Second, our study confirmed previous findings by Mirmirans’ group and ours by instrumental RSD, (i.e., that most of the REM sleep lost in CLI rats was counterbalanced by compensatory NREM sleep during treatment).5,36 Figure 4 displays a large and significant increase of NREM sleep during CLI treatment (from an increase of 45.1% on the first day to 23.3% on the last day of treatment). In contrast, compared to the reduction of REM sleep during RSD, the increase of wakefulness was considerably smaller (Figure 6). To our knowledge, this is the first quantitative report of an NREM sleep increase during postnatal CLI treatment. Figure 5 further demonstrates the relationship between the change of REM sleep and NREM sleep and indicates that NREM sleep always plays a complementary role in the regulation of homeostasis. This finding might indicate that total sleep is strongly protected or else the development of the neuronal structures responsible for wake generation lag significantly behind the development of those for sleep. The later is supported the findings that the orexin-A and -B-like immunopositive cells and fibers are not able to be detected before P15.44 CL in adults, however, decreases REM sleep, increases wakefulness, or shifts deep NREM sleep (stage 3 and 4) to light NREM sleep (stage 1 and 2) or wakefulness, and increases restlessness. For instance, ventromedial hypothalamic superfusion of CLI in adult rats produces a significant reduction of REM sleep (1.0% in CLI group compared to 6.6% in control group) and total sleep (16.4% in CLI group compared to 67.0% in control group).10 In adult humans, CLI significantly reduces REM sleep and increases the number of waking episodes and waking time (11, 45). In adult humans, CLI administration also shifts deep NREM sleep (slow wave sleep stage 3 and 4) to light NREM sleep (stage 1 and 2) and increases intra-sleep restlessness.46,48 Findings of instrumental sleep deprivation in adults and postnatal rats also demonstrate results similar to those which accompany CLI treatment. Long-term RSD by the disk-over-water method results in1 an almost complete removal of REM sleep, 2) about 19% total sleep reduction,52 and 3) a shift from high amplitude NREM sleep to lower amplitude NREM sleep in adult rats. Instrumental RSD via the soft head-plug method reveals significant REM sleep loss without a significant total sleep change in rats at the age of two weeks and with a small total sleep reduction at age three weeks.36 It has been recognized that the rate of PMA is higher during REM sleep than during NREM sleep in postnatal rats.4 The fact that NREM sleep rather than wakefulness compensates for REM sleep loss suggests a net loss of overall stimulation. The fact that REM sleep percentage is two to three times higher in postnatal rats than in adult rats18,49 makes the discovery that NREM sleep compensates for REM sleep loss even more significant and further supports our hypothesis that postnatal RSD mediates the adult depressive behaviors produced by postnatal treatment with CLI and other RSD drugs. Findings produced by instrumental RSD are comparable to those of pharmacological RSD, particularly those achieved with CLI, and suggest that the production of postnatal RSD without increasing wakefulness might be crucial to the resolution of the aforementioned paradox.

Our third finding is that the REM rebound manifests at P19, the first day of recovery (Figure 3), which is consistent with results produced via instrumental RSD. In a two-day period of instrumental RSD started at P12-13, P18, and P26, post-RSD REM rebound emerged in the study of ages P20 and P28 but not P15.36
cate that the effective treatment window for CLI to produce sexual behavior deficits is not later than the age of P14-20. The findings in the present study support the hypothesis that, in rats, a REM sleep homeostatic process develops between two and four weeks of age. A previous publication by Mirmiran and colleagues reported that no REM rebound was found after two weeks of treatment with CLI (25-30 mg/kg). The inconsistency might result from 1) the short-term (one to three hours) recording used in their study and 2) the lower dose of treatment. In addition, we found a sharp decrease of NREM sleep on the first day of recovery as compared with the last day of treatment in experimental rats (Figure 4). This may result from homeostatic regulation during a strong REM rebound on the first recovery day. These findings are also consistent with our results from instrumental RSD studies, in which the percentage of NREM sleep on the first day of recovery was also less than that of two days of RSD. This phenomenon indicates that the homeostasis of total sleep matures at a much earlier stage in development.

RSD, by either instrumental or pharmacological methods, produces dramatic REM sleep reduction during the treatment period. However, REM rebound was not observed until P19 (in the current study), and higher rebound was observed on P28. These results might indicate that REM homeostasis develops during the period of two to four weeks of age. This feature was consistent with other features of REM sleep, such as daily REM sleep percentage, daily number of REM sleep episodes, REM sleep latency, sleep onset REM sleep periods, and mean REM sleep duration in the developmental course. The characteristics of immature REM sleep during the ages of two to four weeks may help to explain why CLI treatment during P14–P20 is more effective in producing adult behavioral abnormalities.

Speculation

The early-age sleep patterns of mammals in general, and humans in particular, are characterized by a very high percentage of REM sleep during which very frequent phasic muscle twitching occurs. The number of phasic events dramatically decreases as animals mature. This endogenously generated self-stimulation is possibly the driving force behind development according to the hypothesis of activity dependence. Findings of the current study—that postnatal RSD induced by treatment with CLI as well as that induced by shaking produce considerable REM sleep reduction without a proportional increase in wakefulness—suggest a net loss of stimulation during treatment. The net loss of stimulation resulting from the reduction of endogenous (self) stimulation generated during REM sleep without compensation by an increase of exogenous stimulation via behavioral movement during waking periods might account for the causative pathogenesis of adult depressive behavior.

Conclusion

The finding that CLI reduces REM sleep but not PMA-NS indicates that PMA-NS is part of NREM sleep, and the finding that CLI markedly reduces REM sleep without a proportional increase in wakefulness is possibly the most significant difference between CLI treatment in neonates and in adults. The latter finding implicates that the delayed development of wakefulness possibly responds to the paradox that postnatal RSD produces depression and adult RSD alleviates depression.

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