

Instrumental REM Sleep Deprivation in Neonates Leads to Adult Depression-like Behaviors in Rats

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Study Objectives: Previous studies have demonstrated that neonatal suppression of rapid eye movement (REM) sleep by pharmacologic agents, particularly clomipramine, produces adult depressive behavior. These findings suggest the hypothesis that REM sleep deprivation (RSD) mediates the depressogenic behaviors of neonatally administered antidepressant drugs. Drug suppression of RSD, however, was thought to be confounded by the other effects of the drugs. The current study was aimed to show the adult effect of neonatal RSD in rats by instrumental means, ie, a computer-controlled shaking method.

Design: Three treatment groups were studied: an instrumental RSD group, a yoked control group, and a nonshaken, maternally separated, control group. All treatments began at the age of 14 days and lasted for 7 days. Adult behavior measurements including tests of sexual activity, locomotor activity, shock-induced fighting, and sleep recording were subsequently performed.

Measurements and Results: The major findings of our investigation were that rats subjected to neonatal instrumental RSD demonstrated diminished sexual activity, decreased aggressive behavior, increased percentage of REM sleep, and decreased wake-REM sleep ratio compared with yoked control rats. These data are compatible with the findings from adult rats subjected to neonatal treatment with the REM-sleep suppressant, clomipramine, and supports the hypothesis that neonatal RSD results in adult depressive abnormalities.

Conclusion: Neonatal RSD induced by a nondrug method results in adult depression-like changes similar to those induced by a REM-sleep suppressant drug, although the extent of these changes varies.

Key Words: Ontogeny, neonatal rats, neonatal REM sleep deprivation, REM sleep deprivation, sexual behavior, endogenous depression.

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INTRODUCTION

THE FUNCTION OF NEONATAL RAPID EYE MOVEMENT (REM) SLEEP HAS ATTRACTED CONSIDERABLE ATTENTION SINCE REM SLEEP WAS FIRST OBSERVED IN CHILDREN.¹ A high percentage of REM sleep and a high level of activity, which occurs during REM sleep, are typical features in the early lives of human and altricial mammals.^{2,3} Roffwarg et al first postulated that neonatal REM sleep might play a significant role in development.³ Neonatal suppression of REM sleep by administering clomipramine (CLI), an antidepressant drug, to rats from postnatal days (PN) 8 to 21 resulted in adult rats with more REM sleep and less sexual activity.⁴ Vogel and Vogel interpreted these changes as depressive signs and hypothesized the CLI rat to be a model of endogenous depression.⁵ Vogel also discussed the similarity of endogenous depression with Major Depressive Disorders with Melancholic Feature listed in the DSM IV.^{6,7} Since then, extensive studies have been performed in Vogel's as well as others' laboratories based on this model. Major findings from CLI rats in comparison with the saline-treated control rats using behavior measurement are (1) decreased sexual activity,^{4,8-10} (2) decreased aggression as measured by shock-induced fighting,¹¹ (3) increased locomotor activity,¹²⁻¹⁴ (4) decreased pleasure-seeking behaviors as measured by intracranial self-stimulation,¹⁵ (5) increased immobility,^{16,17} and (6) increased alcohol intake.¹⁷⁻¹⁹ Rapid eye movement sleep abnormalities found in CLI rats include increased tonic and phasic REM sleep and decreased REM latency.^{4,20} Several other antidepressant drugs, when administered to neonatal rats, also produce adult signs of depression. Neonatal treatment with

desipramine, zimeldine, and Lu 10-134-C have produced increased immobility time in the forced swim test^{21,22} and treatment with nomifensine has increased adult alcohol consumption.^{17,23} These drugs have different aminergic effects. Clomipramine preferentially but not selectively blocks norepinephrine and serotonin (5-HT) uptake; clonidine is an α_2 agonist; nomifensine blocks both dopamine and 5-HT uptake; and Lu 10-134-C also blocks 5-HT uptake.^{22,24,25} Regardless of their aminergic effects, however, all tested antidepressant drugs that produce adult depressive behavior have RSD effects.²⁴ These facts suggested Vogel's hypothesis that neonatal RSD by these drugs, rather than their different aminergic effects, mediates adult depression (for review see^{7,26}). Consistent with this hypothesis, iprindole, an antidepressant drug that does not decrease REM sleep, does not produce adult depressive symptoms following administration to neonatal rats.²⁷

The above findings strongly support the hypothesis that neonatal RSD mediates the depressogenic effects of neonatally administered antidepressant drugs. However, RSD by drugs was thought to be confounded by the other effects of drugs. A test of this hypothesis requires a method in which the effects of RSD are not confounded by drug effects. In order to accomplish this end, we conducted the following experiment using our newly developed, non-drug-induced method of neonatal RSD.²⁸

METHODS

Animals

All procedures were approved by the Institutional Animal Care and Use Committee of Emory University School of Medicine.

Male, neonatal, Long-Evans, hooded rats were studied. Pregnant rat mothers were obtained from a commercial supplier, and all neonatal rats were born in our laboratory. The neonatal rats remained with their dams until PN 13.

Design

Three treatment groups were tested. On PN 13, all rats were weighed, numbered, and assigned to 1 of 3 groups, including an instrumental RSD group (RSD rats), a yoked control group (YC), and a nonshaken and

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maternally separated control group (MS, 4 peers per cage). Then, recording electrodes were surgically implanted in RSD rats and YC rats by the soft head-plug method.²⁹ Rat pups were not cross-fostered before being assigned to each groups because the capacity in running the experiment limits the rats to a small number. However, rat pups in each group of the same "RUN" were picked from the same litter with matched body weight. Since normal sleep recording in the neonatal rat from PN 14 to PN 26 had been done previously,³⁰ sleep recording was not performed in the MS rat because the MS rats were treated in conditions similar to those in the previous study. Therefore, MS rats had only a sham operation with the opening and closing of their skin. After surgery, all rat pups were separated from their dams. The RSD and YC rats were housed individually in their recording chambers for adaptation to recording conditions. The MS rats were housed in a group. After 24 hours of adaptation, polysomnographic recording and instrumental RSD were begun and continued for 7 days. A thermal-neutral ambient temperature for rats at this age³¹ was maintained at 27°C to 28°C by a temperature controller that operated infrared heating lights. The rats were fed manually with fresh formula (0.2 ml - 1.5 ml depending upon age) generally used for human infants every 4 hours via a feeding needle. The formula was also accessible inside the cage. The study was conducted following a 12:12 hour light-dark cycle. After treatment, all electrodes (in RSD and YC rats) were removed, and rats of all groups were group housed in a regular cage (2-4/cage) under standard conditions required by the Institutional Animal Care and Use Committee of Emory University.

At 3 months of age, rat behaviors were measured, including sexual, locomotor, and open-field activity. Thereafter, electrodes were implanted under Nembutal anesthesia (50 mg/kg) to measure adult electroencephalogram (EEG), electromyography (EMG), and theta rhythm in all groups of rats. Three days of polysomnographic recording were conducted after 10 days of recovery and adaptation. This was followed by the measurement of aggressive behavior.

Polysomnographic Recording and Instrumental RSD in Neonatal Rats

Electrode Implantation

At PN 13, RSD and YC rats underwent electrode implantation by the soft head-plug method under metofane anesthesia.²⁹ The EEG electrode was a 20-cm length of thin but strong Teflon-coated wire. The wire was led by a suturing needle through the rat's soft skull to the epidural space, was exited through the skull, and finally was fastened to the entering end. Another micro-wire was implanted in the same way 2 mm to 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 muscle for EMG recording. All wires exited the skin through a soft guide tube and were connected to a small suspending connector. The skin was carefully closed with a silk suture, and the guide tube containing the wires was secured to the skin.

Sleep Recording and RSD

The methods of polysomnographic recording and RSD in neonatal rats have been reported.²¹ Each RSD and YC rat was singly housed in a Plexiglas chamber maintained at 27°C to 28°C, and the pair was separated by a vertical wall. A suspended formula holder (height, 2.0 cm; diameter, 2.0 cm) was hung in each side of the cage to make fresh formula accessible throughout the shaking period. The floor of the housing chamber was the horizontal surface of a general laboratory shaker (82889-ID, Thomas Scientific). Continuous (24 hour/day) polysomnographic EEG and EMG recordings of the RSD and YC rats were made throughout the RSD periods. Paper speed was 50 mm per minute to 60 mm per minute. Physiologic signals of EEG and EMG were also sent to a data-collection board (Dap3000a/212, Microstar Lab, Bellevue, WA, USA) by which the signal was processed for online sleep recognition and storage. The RSD was accomplished instrumentally by a computer-controlled shaking method. Each time a REM sleep onset was detected

from the RSD rat, the computer turned on the shaker for 5 seconds, thereby oscillating the chamber that housed the RSD and YC rats. The YC rat might accordingly obtain REM sleep, while the RSD rat was in awake or in non-REM (NREM) sleep. Previous work has found that the efficacy of the REM-sleep termination was positively related to oscillation speed. At the beginning of the RSD period, and periodically thereafter, the experimenter adjusted the shaker speed of each RSD rat to ensure that the majority of stimulation (shaking episodes) was able to terminate REM-sleep episodes of the RSD rat.

Sleep Scoring

We found that computer scoring of polysomnographic data collected during neonatal RSD was not satisfactory because the motion of the cable during chamber oscillation (RSD) generated some artifacts. However, this was not the case in the adult sleep recording because these rats were not subjected to instrumental RSD (see below). Thus, recordings from neonatal rats were scored visually in 30-second epochs using the previously established criteria, ie wakefulness was indicated by low EEG and high EMG, REM sleep was indicated by low EEG and low EMG, and NREM sleep was indicated by high EEG and low EMG.^{30,32}

Polysomnographic Recording in Adult Rats

Surgical Electrode Implantation

The standard of surgical electrode implantation for polysomnographic recording in adult rats has been established in previous studies.^{33,34} Rats underwent electrode implantation at the age of 3 to 4 months after tests of sexual behavior, locomotor activity, and open field activity. Under Nembutal anesthesia (50mg/kg, intraperitoneal), 4 screws reaching to the epidural space were implanted in the skull for recording EEG and theta rhythms. Two pieces of microspring (outside diameter, 1 mm) connected to 1-inch-long stainless still wire were sutured on the neck muscle for EMG recording. The screws, which served as recording electrodes, were connected by short wires to a small connector that was cemented on the skull. The skin was then closed surrounding the connector.

Sleep Recording and Scoring

The rats were allowed to recover from the operation for 2 weeks; thereafter, polysomnographic recording began and continued for 3 days. The EEG, EMG, and hippocampal theta electrodes were connected to a polysomnographic machine, and amplified signals were fed a computer. Polysomnographic recordings in adult rats were computer scored. The computer program used has been described in previous publications.^{30,32-34} Briefly, the program calculated the mean power of EEG, EMG, and theta rhythms in each 30-second epoch and compared these amplitudes to reference EEG and EMG amplitudes of each sleep-wake state. A combination of low EEG, high EMG, and low theta rhythm was scored as waking; low EEG, low EMG, and high theta rhythm was scored as REM sleep; high EEG, low EMG, and low theta rhythm was scored as NREM sleep. A quarter of computer scored results were visually cross-checked with paper recordings.

Behavioral Tests

Sexual Behavior

Methods of the sexual behavior test have been described previously.¹⁰ Three- to 4-month old female Long-Evans rats were obtained from a commercially available source, and their ovaries were removed under Nembutal anesthesia (50 mg/kg) 1 week to 10 days prior to testing. Female receptivity was introduced by administering estradiol and progesterone prior to testing. The sexual behaviors of male rats were measured in 30-minute test sessions. For habituation, males were placed in a clear plastic chamber 5 minutes before receptive females were intro-

duced. Each female was paired with a different male. Testing was done for 30 minutes in the dark with dim-red-light illumination. Observers were blind to rat treatment, and they recorded number of mounts, intromissions, ejaculation, latency to first mount, latency to first ejaculation, and pause after first ejaculation to next mount. The bedding of wood shavings on the floor was changed after each testing session. The test was performed once a week for 3 weeks. In order to rule out first-time effects, only the results of the second and third testing sessions were used. Data were presented as the mean of the 2 tests.

Locomotor Activity Detection and Open Field Test

Total (vertical and horizontal) activities were automatically detected by a device of Digiscan (RXY 8, Omnitech Electronics, Inc. Columbus, OH) and have been described elsewhere.¹² Each rat was placed in a chamber (15.5" x 15.5") for 8 minutes. The chamber was lit by a 15-watt red light 40 cm above the center. Rats were tested in the chamber for a total of 8 minutes in the dark phase of the 12:12-hour light-dark cycle. Total activity and ambulating activity during 2-minute intervals were recorded manually. Three consecutive daily sessions were recorded.

Open field activity was tested in a cylindrical open field chamber. The circular floor was 70 cm in diameter, and the walls were 30 cm high. The floor was divided into 3 concentric circles with radial sectors. The chamber was lit by a 75-watt white light 90 cm above the center. Rats were tested in the chamber for 8 minutes in the dark phase of the 12:12-hour light-dark cycle. For testing, a rat was placed in the inner circle. Its movements to other sectors and time in each sector were recorded by an observer blinded to rat treatment. The floor was wiped clean after each rat was tested. Typically, rats were tested over 3 consecutive days. In order to separate the activity that could possibly reflect anxiety, adaptation to a novel environment, and generally active movement, cumulative locomotor activity was separated into the first 2 minutes and the next 6 minutes of each session.

Aggressive Behavior

Shock-induced fighting behavior was tested by observing the offen-

sive and defensive responses of pairs of experimental and control rats.¹¹ Prior to testing, all rats were paired by body weight (within 15 g) to diminish size differences that could affect the behavioral results. Tests were performed daily for 4 days (3 real tests). On the first day, animal pairs were placed in the chamber for a 12-minute habituation period. On days 2 to 4 (called test days 1 to 3), the session was started with a 2-minute habituation period followed by 10 minutes of intermittent shock delivery. Shocks (1.33 mA, 0.5-second duration) were delivered to a grid-floor animal test cage (E10-10SF, Coulbourn Instruments, Coulbourn, Penn) on a randomly generated variable schedule with a minimum 5-second and a maximum 10-second intershock interval. The shock current was generated by a grid-floor shocker (E13-08, Coulbourn Instruments, Coulbourn, Penn) and controlled by a program via the hardware used for sleep deprivation. This resulted in a total of 70 to 80 shocks within the 10-minute session. Offensive and defensive behaviors, including upright positioning, leaping, mounting, crouching, and supine positioning were scored using an observation system that was devised based on a description of aggressive-behavior topography for rats. Total offensive behavior and defensive behavior was subsequently calculated.

Data Analysis and Statistics

The entire set of adult measurements and tests required a great deal of time. Depending on the age of the rats, different measurements and tests might be alternatively done on different RUNs, ie most of the rats did not experience the entire set of measurements and tests. Data of each variable presented were compiled from all rats selected to undergo that measurement or test. One exception was that 1 cage of RSD rats was eliminated before undergoing sexual behavior tests—1 of the RSD rats in that cage was female. Because this study was designed to use all male rats, clearly the female rat was misjudged as a male rat at the beginning of the experiment. In order to avoid the possible effects of the mixed housing of male and female rats on sexual performance, this cage of rats (a total of 3) was eliminated.

In order to compare the sleep data during neonatal RSD in RSD and YC rats with nontreated but MS rats, neonatal sleep data were drawn from a previous study and culled from identical age groups.³⁰

Two-way (treatment and days/tests) analysis of variance (ANOVA) with posthoc pairwise comparisons by the Student-Newman-Keuls method were used to assess the statistical differences depending on the data of tests, including sleep-wake variables, body weight, and all behavior tests. Statistical significance was defined as $P < .05$. All data were presented in the form of mean \pm SE.

RESULTS

Body Weight

Eight rats in each group (2 RUNs) underwent a complete set of body-weight measurements at the time prior to surgery (PN 13), daily during treatment, 1 week after treatment, and at the age of 3 months. Figure 1 demonstrates the changes of body weight on the second day (D2) and the last day (D7) of neonatal RSD (corresponding to PN 14 and PN 19) as well as at the age of 3 months. By the end of the neonatal RSD, i.e., D7, the mean body weights were 32.1 ± 0.6 g, 31.9 ± 0.3 g and 34.6 ± 1.1 g in RSD, YC, and MS rats, respectively. At 3 months of age, the mean body weights in RSD, MS, and YC rats were 436.9 ± 15.2 g, 474.6 ± 9.1 g, and 461.7 ± 14.9 g, respectively. One-way ANOVA showed that differences in body weights between YC, RSD, and MS rats were not significant at any age (Figure 1). However, the P value in comparison of weights on D7 was close to .05 (Treatment: $F = 3.31$, $P = .067$).

Sleep During Neonatal RSD

The percentage of REM sleep, NREM sleep, and waking

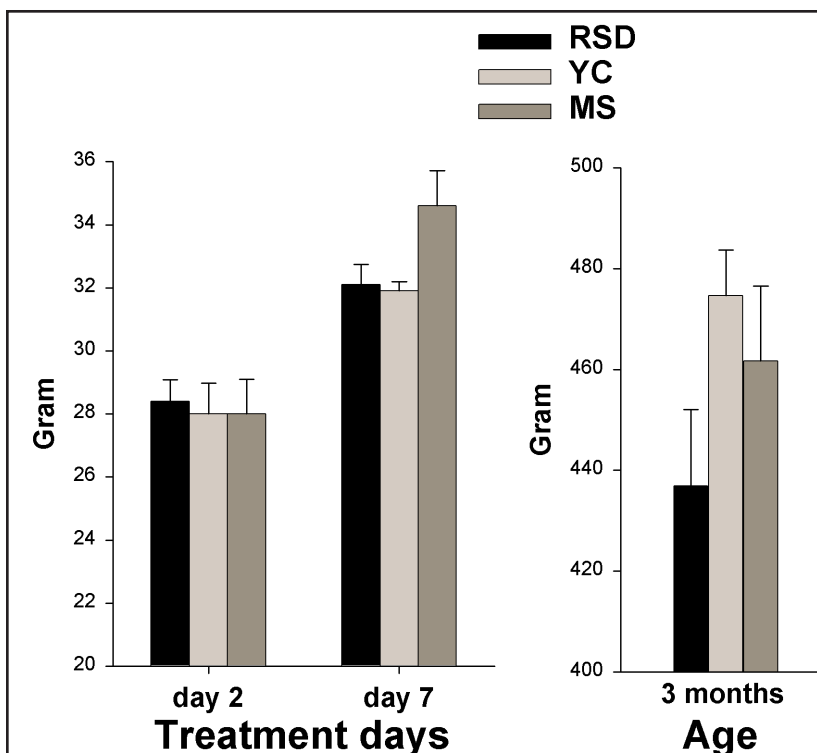
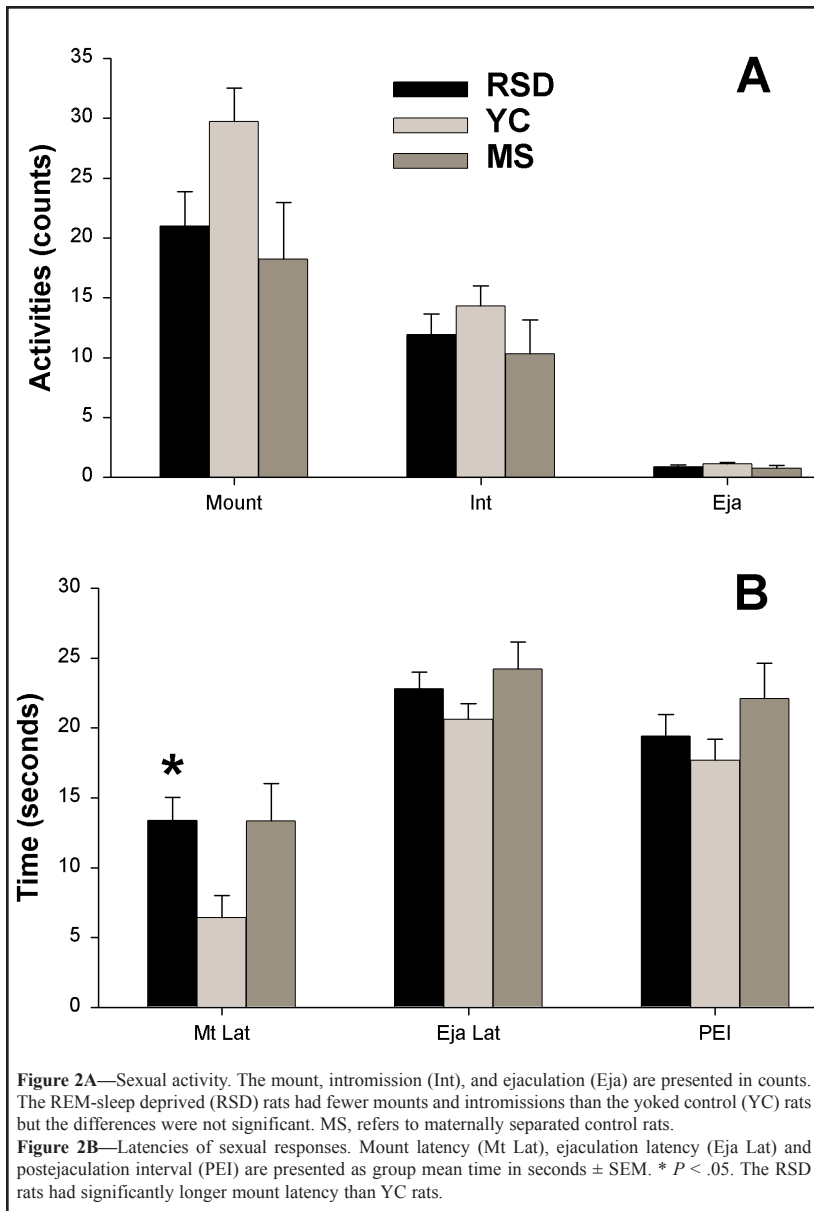


Figure 1—Increases in body weight, in grams, on neonatal treatment day 2 (postnatal day 14) and day 7 (postnatal day 21) and at 3 months of age. Although differences were shown at day 7 and 3 months between groups, the differences were not significant. RSD refers to REM-sleep deprived rats; YC, yoked control rats; MS, maternally separated control rats.



during RSD are shown in Table 1. Nonshaken MS rats ($n=9$) had mean REM sleep of $36.52\% \pm 2.0\%$ on D1 corresponding to the age of P14. Thereafter, REM sleep gradually reduced to 33.26%, 26.57%, and 24.31% on treatment days 3, 5, and 7, corresponding to ages of P16, P18, and P20, respectively. The REM sleep of RSD rats was 9.01%, 11.94%, 7.75%, and 7.75% at days 1, 3, 5, and 7, respectively. The YC rats had REM sleep of 23.1%, 20.18%, 16.83%, and 15.24% on days 1, 3, 5, and 7, correspondingly. The REM sleep of RSD rats during the 7 days of RSD was $51.17\% \pm 7.25\%$ (7.96% of total time) less than that of YC rats and $68.3\% \pm 5.1\%$ (21.04% of total time) less than the REM sleep of MS rats. In addition, YC rats also had a mild REM sleep loss during RSD. Compared with the REM sleep of MS rats, the REM sleep of YC rats was reduced by an average of $37.51\% \pm 1.08\%$ (11.33% of total time). Two-way ANOVA demonstrated that the differences among treatment groups were significant (treatment X day, $F = 4.85$, $P < .0001$, $P < .05$ on multiple pairwise comparisons, see Table 1).

The NREM sleep of RSD rats was significantly increased when compared with that of either YC or MS rats during the entire RSD period (treatment: $F = 261.18$ and $P < .0001$, see Table 1). Corresponding to treatment days 1, 3, 5, and 7, the NREM sleep of RSD rats increased 19.5%, 16.18%, 18.99%, and 11.27% when compared with YC rats, and it increased 92.91%, 80.34%, 65.34%, and 40.51% when compared with MS rats. In addition, the NREM sleep of YC rats was also significantly increased when compared with MS rats. The overall increase was $48.96\% \pm 9.48\%$.

Table 1 also shows that there was a significant but small difference in percentage of time awake among treatment groups (treatment, $F = 11.599$, $P < .0001$, $P < .05$ in all multiple pairwise comparisons). The RSD rats had a mean increase of 5.75% (1.76% of total time) compared with YC rats and a mean decrease of 6.84% (2.61% of total time) compared with MS rats (Table 1). However, YC rats had a moderate decrease rather than an increase in wake time ($11.79\% \pm 1.99\%$, 4.37% of total time) compared with MS rats.

Furthermore, Table 1 displays the increase in wake time as a percentage of REM-sleep loss during the same RSD day, ie, daily $\Delta\text{wake}^+ / \Delta\text{REMs}^-$. Note, a positive value of the ratio

means that the change of wake time was decreased rather than increased. Compared with the YC rat, the increase of wakefulness in RSD rat was only a very small portion of the REM-sleep reductions (-26.52%, 4.7%, 1.25%, and 11.52% at RSD D1, D3, D5, and D7, respectively).

Thus, 7 days of neonatal RSD resulted in a significant decrease of REM sleep, a significant increase of NREM sleep, and a significant but small increase of wakefulness. This resulted in a low ratio of $\Delta\text{wake}^+ / \Delta\text{REMs}^-$.

Adult Testing

Sexual Behavior Test

Sexual behavior was tested in RSD ($n=19$), YC ($n=20$), and MS ($n=7$) groups. Across the 6 measured variables, RSD rats showed lower sexual activity than did YC rats, as indicated by fewer mounts, fewer intromissions,

Table 1—Sleep-wake changes during neonatal REM-sleep deprivation

Treatment Day	Variables	RSD*	YC*	MS*	RSD vs YC†	RSD vs MS†	YC vs MS†
1	REM sleep, %	9.07 \pm 3.4	23.10 \pm 2.6	36.52 \pm 2.0	-60.74‡	-75.16‡	-36.75‡
	NREM sleep, %	58.07 \pm 7.9	48.59 \pm 4.2	30.15 \pm 4.9	19.51‡	92.60‡	61.16‡
	Wake, %	32.86 \pm 6.0	28.30 \pm 2.0	33.34 \pm 3.5	16.11‡	-1.44‡	-15.12‡
	$\Delta\text{Wake}/\Delta\text{REM sleep}, \%$				-26.52	-1.92	41.14
3	REM sleep, %	11.94 \pm 3.6	20.18 \pm 1.1	33.26 \pm 0.8	-40.83‡	-64.10‡	-39.33‡
	NREM sleep, %	54.67 \pm 5.1	47.05 \pm 3.4	30.31 \pm 0.5	16.20‡	80.37‡	55.23‡
	Wake, %	33.40 \pm 3.9%	32.77 \pm 2.5%	36.43 \pm 0.8%	1.92‡	-8.32‡	-10.05‡
	$\Delta\text{Wake}/\Delta\text{REM sleep}, \%$				-4.7	12.98	25.55
5	REM sleep, %	7.75 \pm 2.0	16.83 \pm 2.6%	26.57 \pm 2.4%	-53.95‡	-70.83‡	-36.66‡
	NREM sleep, %	58.35 \pm 4.6	49.03 \pm 3.1	35.29 \pm 3.2	19.01‡	65.34‡	38.93‡
	Wake, %	33.90 \pm 4.2	34.13 \pm 1.3%	38.14 \pm 3.5	-0.67‡	-11.12‡	-10.51‡
	$\Delta\text{Wake}/\Delta\text{REM sleep}, \%$				1.25	15.70	28.67
7	REM sleep, %	7.75 \pm 2.2	15.24 \pm 1.5	24.31 \pm 0.5	-49.15‡	-68.12‡	-37.31‡
	NREM sleep, %	53.40 \pm 2.6	47.99 \pm 3.4	34.15 \pm 0.9	11.27‡	56.37‡	40.53‡
	Wake, %	38.85 \pm 1.4	36.77 \pm 3.3	41.54 \pm 0.5	5.66‡	-6.48‡	-11.48‡
	$\Delta\text{Wake}^+/\Delta\text{REM sleep}^-, \%$				-11.52	9.51	30.77

*Percentage of total 24-hour recording time

†Change of percentage of variable between groups

‡ $P < .05$

§Values of wake to REM-sleep ratio reflect a change of wake as a percentage of REM sleep

REM refers to rapid eye movement, RSD, REM-sleep deprived rats; YC, yoked control rats; MS, maternally separated control rats; NREM, non-rapid eye movement

and fewer ejaculations as well as longer mount latency, longer ejaculation latency, and longer postejaculation interval (Figure 2). However, the difference between RSD and YC rats was significant only in mount latency (treatment: $F = 6.902$ and $P < .0001$, $P < .05$ in RSD vs YC). None of the comparisons in the 6 sexual variables were significant between RSD and MS rats or between YC and MS rats.

Autodetected Locomotor Activity

Overall, total activity (detected by horizontal sensors and vertical sensors) in RSD rats ($n=8$) in both the first 2 minutes and the next 6 minutes was not significantly different from that of either YC ($n=7$) or MS rats ($n=7$). Two-way ANOVA showed that in the Treatment-X-Day anal-

ysis, $F = 0.591$ and $P = .67$ for the first 2 minutes of activity and $F = 0.302$ and $P = .86$ for the next 6 minutes.

Open Field

The mean cumulative values of the first 2 minutes and the next 6 minutes are separated by the inner and outer field in data analysis. Overall, all rats had less activity in the inner field than in the outer field. The RSD rats ($n=8$) did not exhibit any significant difference from other groups (YC, $n=7$; MS, $n=7$) in all measured variables including the first 2 minutes in both the inner (Treatment X day: $F = 0.177$, $P = .95$) and the outer (Treatment X day: $F = 0.476$, $P = .75$) section as well as the next 6 minutes in both the inner (Treatment X day: $F = 0.222$, $P = .92$) and the outer (Treatment X day: $F = 0.208$, $P = .933$) section.

Shock-induced Fighting

Shock-induced fighting tests were performed on paired rats. An RSD rat was paired with a YC rat. Fifteen pairs of RSD-YC rats were tested. Total offensive behavior consisted of mounts, standing upright, leaping, and crouching. Total defensive behaviors consisted of standing upright but standing lower than the fighting partner, crouching, and lying supine. All behaviors were recorded and analyzed. The RSD rats had a mean of 12.6 ± 0.8 offensive behaviors that were significantly less (2-way ANOVA, Treatment, $F = 10.476$, $P = .0017$) than YC rats exhibited (21.6 ± 0.8). Furthermore, RSD rats demonstrated significantly (2-way ANOVA, Treatment: $F = 11.866$, $P = .0009$) more defensive behaviors (19.3 ± 0.74) than YC rats (10.5 ± 0.74) (Figure 3).

Adult Sleep

Following sexual behavior tests, open field tests, and an adequate break (2 weeks), 2 RUNs of rats were polysomnographically recorded for 3 days. The number of rats representing each group was: RSD=8; YC=7; and MS=6. There was no significant difference in NREM sleep (Treatment: $F = 0.959$ and $P = .40$) and total sleep (Treatment: $F = 1.5620$ and $P = .24$) among groups, although RSD rats demonstrated a trend of increasing total sleep; however, REM sleep was significantly increased (Treatment: $F = 5.05$ and $P = .0175$) in RSD rats ($7.27\% \pm 0.27\%$) when compared with YC rats ($6.12\% \pm 0.29\%$) as well as with MS rats ($6.30\% \pm 0.29\%$, see Figure 4). In addition, the wake to REM-sleep ratio in RSD rat was also significantly lower than that in both YC and MS rats (Treatment: $F = 5.50$, $P = .013$). The mean wake to REM-sleep ratio in the RSD rat was 6.17 ± 0.31 , compared with 7.67 ± 0.33 and 7.06 ± 0.33 in YC and MS rats, respectively (Figure 4).

DISCUSSION

Neonatal Treatments and Their Acute Effects

Our study demonstrated a dramatic REM-sleep reduction during neonatal RSD (Table 1). The mean overall REM-sleep reduction in RSD rats was $68.3\% \pm 5.1\%$ compared with MS rats and 51.17% compared with YC rats. These changes are comparable to our finding in pharmacologic RSD with the use

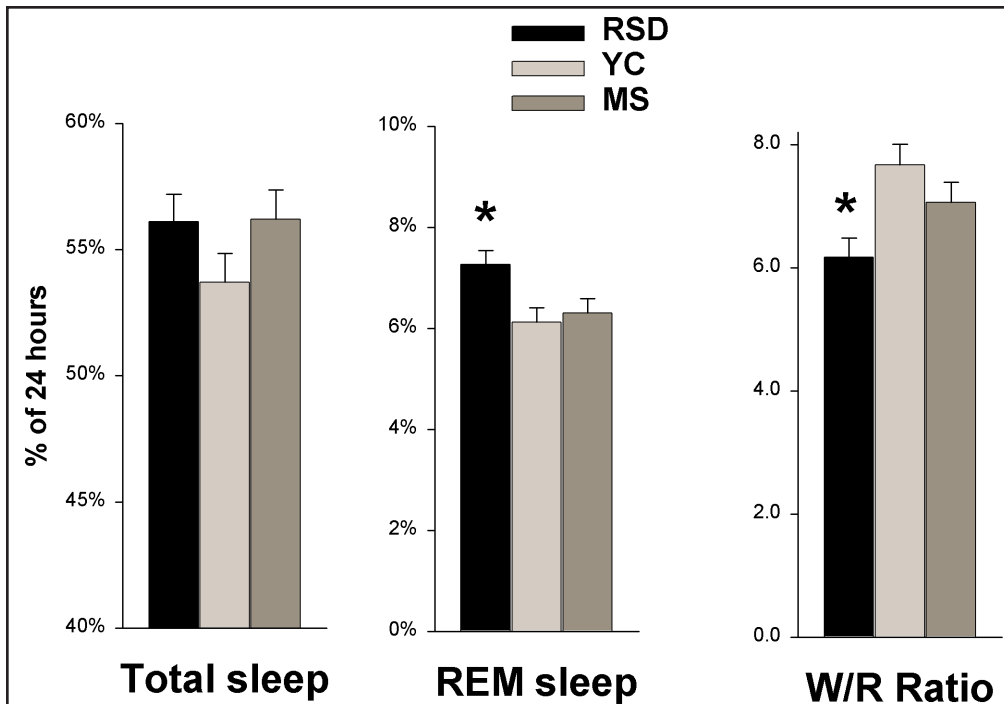


Figure 3—Aggressive behavior measured by shock-induced fighting. Total count of offensive and defensive behaviors, including leaping, standing upright, and crouching, is presented as mean \pm SEM. $*P < .05$. Compared with yoked control (YC) rats, the REM-sleep deprived (RSD) rats exhibited significantly more defensive behavior than YC rats and fewer offensive behaviors than the YC group.

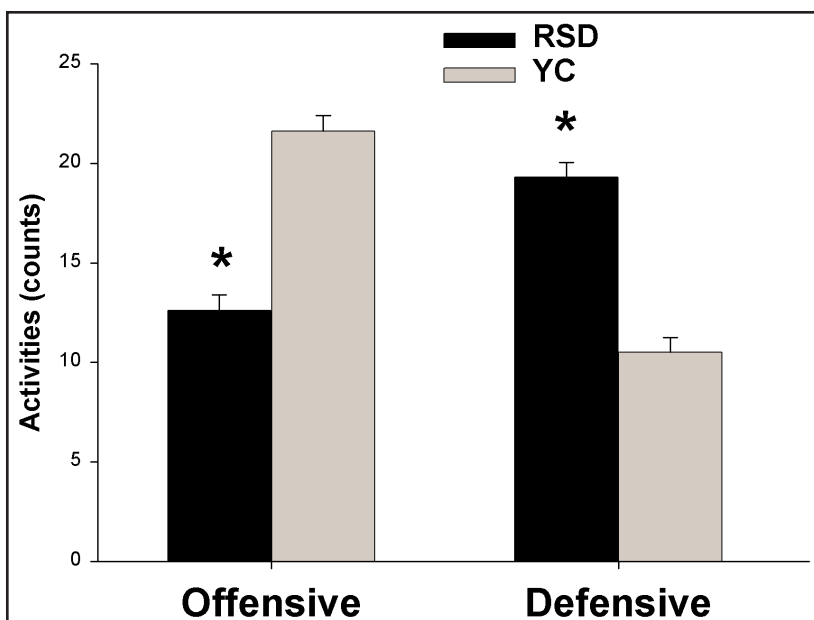


Figure 4—Sleep of adult rats. Both rapid eye movement (REM) and total sleep expressed as a percentage of total recording time (24 hours, mean \pm SEM). $*P < .05$. The percentage of REM sleep in the REM-sleep deprived (RSD) rats was significantly higher than that in yoked control (YC) rats. The RSD rats also had significantly lower wake to REM-sleep (W/R) ratio than that of the YC rats.

of CLI (20mg/kg) at the same age and with a similar treatment period, which had a REM-sleep reduction of $55.8 \pm 7.3\%$.³⁵ These changes are also comparable to the original study conducted by Mirmiran et al.⁴ This correspondence indicates that neonatal RSD by our nonpharmacologic method is as sufficient as the use of CLI in producing neonatal REM-sleep reduction. In addition, NREM sleep rather than wakefulness was found to be significantly increased and compensated for most of the REM-sleep loss during RSD (Table 1). Similar phenomena were also described in neonatal pharmacologic RSD by Mirmiran et al.⁴ and researchers in our laboratories.³⁵ These findings indicate that there is a strong tendency toward sleep homeostasis, that the wake-generation and maintenance system is very immature in this period, or a combination of these factors occurs.

One limitation of the neonatal RSD-by-instrument method is that it is not applicable in rats younger than the age at which NREM sleep is distinguishable by high-amplitude electroencephalography. In general, this age is PN 10 to PN 12.² Thus, neonatal RSD in this study was started at PN 14, which is a week later than the time at which pharmacologic neonatal RSD was instituted in other studies.^{4,8,20} Our recent study showed that 1 week of RSD by the use of CLI started at PN 14 is also effective in producing behavior abnormalities in adult rats. However, the later or the shorter of the RSD-treatment windows results in fewer or decreased effects on the adult behavior.¹⁰

Adult Behavior and Depressive Signs

Our major finding is that, compared to YC rats, RSD rats demonstrated behavior changes that mimicked the neonatal RSD that results from the use of CLI, although the extent was smaller. The changes that RSD rats had include (1) diminished sexual behavior as indicated by a significant increase of mount latency (Figure 2), (2) decreased offensive behavior and increased defensive behavior (Figure 3), and (3) significantly more adult REM sleep (Figure 4). These findings are consistent with results from previous studies of neonatal treatment with CLI.^{4,7,26} However, fewer variables in sexual behavior tests were found to be significantly defective in RSD rats compared with YC rats in the present study than in CLI-treated rats compared with saline-treated rats in the past studies. The shorter and later neonatal RSD (from PN 14 to PN 21) in this study may account for such a difference because previous studies of neonatal RSD by the use of CLI were conducted from PN 8 to PN 21.^{4,8,9} This may be explained at least partially by the fact that adult behavior abnormalities that resulted from the neonatal use of pharmacologic RSD depend upon the dose,³⁶ age, and treatment duration,¹⁰ as mentioned earlier. The current study further supports Vogel's hypothesis that neonatal RSD produces adult depression-like changes and that RSD mediates the depressogenic development.²⁶

The mechanism by which neonatal RSD produces such an alteration in behavior remains to be understood. Our recent studies, including this one, have demonstrated that neonatal RSD, either by the use of CLI or an instrument method, results in a larger reduction in REM sleep with a very small increase in wakefulness (Table 1).^{32,35} Thus, the pathology of neonatal RSD, as compared with adult RSD, may be associated with the alteration of a large REM-sleep loss or a lower change in wakefulness.

Decreased brain levels of 5-HT have been found in adult rats that were treated during the neonatal period with the REM-sleep suppressant CLI³⁷ and in 21-day-old rats that were treated during the neonatal period with clonidine.³⁸ Clinical evidence suggests that depression is associated with deficits of the serotonergic system.³⁹ The crucial issue is how the 5-HT release is decreased. In adults, 5-HT neurons fire at their highest rate during wakefulness, at a lower rate during NREM sleep, and completely cease to fire during REM sleep.^{40,41} The extracellular levels of 5-HT in the dorsal raphe nucleus,⁴² medial medullary reticular formation,⁴³ pedunculopontine and laterodorsal tegmental nuclei,⁴⁴ hippocampus,⁴⁵ and frontal cortex⁴³ exhibit a similar pattern, ie, highest in waking, lower in slow-wave sleep, and lowest in REM sleep. Assuming this is the same in neonatal rats, ie, 5-HT release is lower in REM sleep than in NREM

sleep and wakefulness, neonatal RSD (REM reduction) would result in not a decrease but, rather, an increase of 5-HT release regardless of which states of wakefulness or NREM sleep replace the reduced REM sleep.

The difference of the RSD effects between RSD in neonates and adults is that neonatal RSD does not result in a compatible increase of wakefulness, ie, neonatal RSD turns REM sleep into NREM sleep.³⁵ This finding implicates the idea that the overall production or release of 5-HT during neonatal RSD may be significantly less than during adult RSD because most REM-sleep loss is compensated for by increased wakefulness during the treatment period^{35,46} and 5-HT level is the highest during wakefulness. In addition, brain levels of orexins may also be involved in the development of depression-like behavior. Findings of orexinergic activity being higher during REM sleep than during NREM sleep⁴⁷ suggest that neonatal RSD, which turns REM sleep into NREM sleep, suppresses orexinergic activity. This is supported by our recent study that demonstrated that junior CLI rats have decreased brain levels of orexin B⁴⁸ and that orexins excite 5-HT neurons in the dorsal raphe nucleus.⁴⁹

One of the functions of 5-HT is to provide neurotrophic support to the development of neurons; 5-HT significantly increases the length of the primary process growing out from the cell body, the total length of all processes, the total neurites per cell, the number of branch points per cell, and the branch points on the primary neurite in cultured ventrobasal thalamic neurons from newborn rats.⁵⁰ Consistently, neonatal RSD caused by CLI and other methods has resulted in the decreased brain levels of proteins and DNA¹⁴ and decreased brain levels of total and phosphorylated extracellular signal-related protein kinase.⁵¹

Hence, the above evidence implicates the behavior abnormalities resulting from neonatal RSD as being associated with the fact that neonatal RSD deprives neonatal REM sleep without correspondingly increasing wakefulness. This is likely to result in a down regulation of orexinergic and 5-HT activity and a subsequent effect of a reduced neurotrophic support during the critical developmental period. The mechanism responsible for neonatal RSD failing to increase wakefulness is not known. One possibility is that the wake-generation system is immature. This view is supported by the finding that orexin-A-like and orexin-B-like immunopositive cells and fibers are not detected from PN 0 to PN 10 and are very weak on PN 15.⁵² However, the direct evidence of RSD leading to depression in neonates and improving depression in adults remains to be discovered.

Implications of Adult Sleep Change

The finding that the wake to REM-sleep ratio was significantly lower in the RSD rats than in both YC and MS rats indicates that the change of the wake to REM-sleep ratio is more sensitive than is REM sleep to neonatal RSD. Figure 4 displays a trend of both increasing REM sleep and decreasing wakefulness in RSD.

This is consistent with the effect of 5-HT and orexins on simultaneously promoting wakefulness and suppressing REM sleep and is supported by our preliminary findings that neonatal RSD by CLI results in decreased orexin B levels in multiple brain regions⁴⁸ and by the report of Mavanji and Datta that neonatal treatment with CLI results in unregulated cholinergic activity.⁵³

Our second finding is that RSD rats are actually not depressive when compared to MS rats. The YC rats did indeed exhibit behavior changes toward the opposite direction in several variables, including aggression, sexual activity, and locomotor activity when compared to MS rats, but the differences were not significant. These results indicate that the shaking as a nonspecific factor may produce effects that counter the effect of neonatal RSD and that neonatal RSD-induced behavior abnormalities in the adult may be attenuated by simultaneous RSD with shaking stimulation. One may think that no implantation of real electrodes in the MS rats may produce fewer long-term effects on the development; however, this theory needs to be tested.

The current study indicates that experiments in rats with neonatal RSD caused by nonpharmacologic means produce findings consistent with those from rats with RSD caused by pharmacologic methods, such as by the administration of CLI. These findings support our hypothesis that neonatal RSD produces adult depression-like changes.

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REFERENCES

- Asrenskey E, Kletement N. Eye movement during sleep. *Fed Proc* 1953;12:6-7.
- Jouvet-Mounier D, Astic L, Lacote D. Ontogenesis of the states of sleep in rat, cat, and guinea pig during the first postnatal month. *Dev Psychobiol* 1970;2:216-39.
- Roffwarg HP, Muzio JN, Dement WC. Development of the human sleep-dream cycle. *Science* 1966;152:604-19.
- Mirmiran M, van de Poll NE, Corner MA, van Oyen HG, Bour HL. Suppression of active sleep by chronic treatment with chlorimipramine during early postnatal development: effects upon adult sleep and behavior in the rat. *Brain Res* 1981;204:129-46.
- Vogel GW, Vogel FA. A new animal model of human endogenous depression. *Sleep Res* 1982;11:222.
- Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Text version (DSM-IV-TR). Washington, DC: American Psychiatric Association; 2000.
- Vogel G, Neill D, Hagler M, Kors D. A new animal model of endogenous depression: a summary of present findings. *Neurosci Biobehav Rev* 1990;14:85-91.
- Neill D, Vogel G, Hagler M, Kors D, Hennessey A. Diminished sexual activity in a new animal model of endogenous depression. *Neurosci Biobehav Rev* 1990;14:73-6.
- Velazquez-Moctezuma J, Aguilar-Garcia A, Diaz-Ruiz O. Behavioral effects of neonatal treatment with clomipramine, scopolamine, and idazoxan in male rats. *Pharmacol Biochem Behav* 1993;46:215-7.
- Feng P, Ma Y, Vogel GW. The critical window of brain development from susceptible to insusceptible. Effects of clomipramine neonatal treatment on sexual behavior. *Brain Res Dev Brain Res* 2001;129:107-10.
- Vogel G, Hartley P, Neill D, Hagler M, Kors D. Animal depression model by neonatal clomipramine: reduction of shock induced aggression. *Pharmacol Biochem Behav* 1988;31:103-6.
- Hartley P, Neill D, Hagler M, Kors D, Vogel G. Procedure- and age-dependent hyperactivity in a new animal model of endogenous depression. *Neurosci Biobehav Rev* 1990;14:69-72.
- Hilakivi LA, Taira T, Hilakivi I, MacDonald E, Tuomisto L, Hellevuo K. Early postnatal treatment with propranolol affects development of brain amines and behavior. *Psychopharmacology (Berl)* 1988;96:353-9.
- Mirmiran M, Scholtens J, van de Poll NE, Uylings HB, van der Gugten J, Boer GJ. Effects of experimental suppression of active (REM) sleep during early development upon adult brain and behavior in the rat. *Brain Res* 1983;283:277-86.
- Vogel G, Neill D, Hagler M, Kors D, Hartley P. Decreased intracranial self-stimulation in a new animal model of endogenous depression. *Neurosci Biobehav Rev* 1990;14:65-8.
- Velazquez-Moctezuma J, Diaz Ruiz O. Neonatal treatment with clomipramine increased immobility in the forced swim test: an attribute of animal models of depression. *Pharmacol Biochem Behav* 1992;42:737-9.
- Hilakivi LA, Taira T, Hilakivi I, Loikas P. Neonatal treatment with monoamine uptake inhibitors alters later response in behavioural 'despair' test to beta and GABA-B receptor agonists. *Pharmacol Toxicol* 1988;63:57-61.
- Dwyer SM, Rosenwasser AM. Neonatal clomipramine treatment, alcohol intake and circadian rhythms in rats. *Psychopharmacology (Berl)* 1998;138:176-83.
- Hilakivi LA, Sinclair JD, Hilakivi IT. Effects of neonatal treatment with clomipramine on adult ethanol related behavior in the rat. *Brain Res* 1984;317:129-32.
- Vogel G, Neill D, Kors D, Hagler M. REM sleep abnormalities in a new animal model of endogenous depression. *Neurosci Biobehav Rev* 1990;14:77-83.
- Hilakivi LA, Hilakivi I. Increased adult behavioral 'despair' in rats neonatally exposed to desipramine or zimeldine: an animal model of depression? *Pharmacol Biochem Behav* 1987;28:367-9.
- Hansen HH, Sanchez C, Meier E. Neonatal administration of the selective serotonin reuptake inhibitor Lu 10-134-C increases forced swimming-induced immobility in adult rats: a putative animal model of depression? *J Pharmacol Exp Ther* 1997;283:1333-41.
- Hilakivi LA, Hilakivi I, Ahtee L, Haikala H, Attila M. Effect of neonatal nomifensine exposure on adult behavior and brain monoamines in rats. *J Neural Transm* 1987;70:99-116.
- Vogel GW, Buffenstein A, Minter K, Hennessey A. Drug effects on REM sleep and on endogenous depression. *Neurosci Biobehav Rev* 1990;14:49-63.
- Potter WZ, Rudorfer MV, Lane EA. Active metabolites of antidepressants: pharmacodynamics and relevant pharmacokinetics. *Adv Biochem Psychopharmacol* 1984;39:373-90.
- Vogel GW. REM sleep deprivation and behavioral changes. In: Mallick BN, Inoue S, eds. *Rapid Eye Movement Sleep*. London: Narasa Publishing House; 1999:355-66.
- Vogel G, Hagler M. Effects of neonatally administered iprindole on adult behaviors of rats. *Pharmacol Biochem Behav* 1996;55:157-61.
- Feng P, Vogel GW, Obermeyer W, Kinney GG. An instrumental method for long-term continuous REM sleep deprivation of neonatal rats. *Sleep* 2000;23:175-83.
- Feng P, Vogel GW. A new method for continuous, long-term polysomnographic recording of neonatal rats. *Sleep* 2000;23:9-14.
- Vogel GW, Feng P, Kinney GG. Ontogeny of REM sleep in rats: possible implications for endogenous depression. *Physiol Behav* 2000;68:453-61.
- Vogel GW, Feng P. A reply to Frank and Heller about neonatal active sleep. *Sleep* 2000;23:1005-14.
- Feng P, Ma Y, Vogel GW. Ontogeny of REM rebound in postnatal rats. *Sleep* 2001;24:645-53.
- Feng PF, Bergmann BM, Rechtschaffen A. Sleep deprivation in rats with preoptic/anterior hypothalamic lesions. *Brain Res* 1995;703:93-9.
- Feng PF, Shaw P, Bergmann BM, et al. Sleep deprivation in the rat: XX. Differences in wake and sleep temperatures during recovery. *Sleep* 1995;18:797-804.
- Feng P, Ma Y. Clomipramine suppresses postnatal REM sleep without increasing wakefulness: implications for the production of depressive behaviors. *Sleep* 2002;25:177-84.
- Vogel G, Hagler M, Hennessey A, Richard C. Dose-dependent decrements in adult male rat sexual behavior after neonatal clomipramine treatment. *Pharmacol Biochem Behav* 1996;54:605-9.
- Feenstra MG, van Galen H, Te Riele PJ, Botterblom MH, Mirmiran M. Decreased hypothalamic serotonin levels in adult rats treated neonatally with clomipramine. *Pharmacol Biochem Behav* 1996;55:647-52.
- Thomas AJ, Erokwu BO, Yamamoto BK, Ernsberger P, Bishara O, Strohl KP. Alterations in respiratory behavior, brain neurochemistry and receptor density induced by pharmacologic suppression of sleep in the neonatal period. *Brain Res Dev Brain Res* 2000;120:181-9.
- Owens MJ, Nemeroff CB. The serotonin transporter and depression. *Depress Anxiety* 1998;8 Suppl 1:5-12.
- Hobson JA, McCarley RW, Wyzinski PW. Sleep cycle oscillation: reciprocal discharge by two brainstem neuronal groups. *Science* 1975;189:55-8.
- McGinty DJ, Harper RM. Dorsal raphe neurons: depression of firing during sleep in cats. *Brain Res* 1976;101:569-75.
- Portas CM, Bjorvatn B, Fagerland S, et al. On-line detection of extracellular levels of serotonin in dorsal raphe nucleus and frontal cortex over the sleep/wake cycle in the freely moving rat. *Neuroscience* 1998;83:807-14.
- Blanco-Centurion CA, Salin-Pascual RJ. Extracellular serotonin levels in the medullary reticular formation during normal sleep and after REM sleep deprivation. *Brain Res* 2001;923:128-36.
- Strecker RE, Thakkar MM, Porkka-Heiskanen T, Dauphin LJ, Bjorkum AA, McCarley RW. Behavioral state-related changes of extracellular serotonin concentration in the pedunculopontine tegmental nucleus: a microdialysis study in freely moving animals. *Sleep Res Online* 1999;2:21-7.
- Park SP, Lopez-Rodriguez F, Wilson CL, Maidment N, Matsumoto Y, Engel J, Jr. In vivo microdialysis measures of extracellular serotonin in the rat hippocampus during sleep-wakefulness. *Brain Res* 1999;833:291-6.
- Kushida CA, Bergmann BM, Rechtschaffen A. Sleep deprivation in the rat: IV. Paradoxical sleep deprivation. *Sleep* 1989;12:22-30.
- Kiyashchenko LI, Milevskiy BY, Maidment N, et al. Release of hypocretin (orexin) during waking and sleep states. *J Neurosci* 2002;22:5282-6.
- Feng P. Brain levels of orexin B are decreased in junior rats neonatally treated with REM sleep suppressant of clomipramine. *Sleep* 2003;26: A35-A36.
- Liu RJ, van den Pol AN, Aghajanian GK. Hypocretins (orexins) regulate serotonin neurons in the dorsal raphe nucleus by excitatory direct and inhibitory indirect actions. *J Neurosci* 2002;22:9453-64.
- Lotto B, Upton L, Price DJ, Gaspar P. Serotonin receptor activation enhances neurite outgrowth of thalamic neurones in rodents. *Neurosci Lett* 1999;269:87-90.
- Feng P, Guan Z, Yang X, Fang J. Changes of Sexual Activity, ERK, pERK, PP1 and MPK-2 in Rat Model of Depression. *Sleep* 2002;25:A17-8.
- Yamamoto Y, Ueta Y, Hara Y, et al. Postnatal development of orexin/hypocretin in rats. *Brain Res Mol Brain Res* 2000;78:108-19.
- Mavanji V, Datta S. Clomipramine treatment in neonatal rats alters the brain acetylcholinesterase activity in adulthood. *Neurosci Lett* 2002;330:119-21.