INTRODUCTION

PREVIOUS STUDIES FOUND THAT IN ALTRICIAL MAMMALS, NEONATES HAD MUCH MORE REM SLEEP THAN ADULTS. For example, in rats, one-week-old neonates had about nine times more REM sleep per 24 hours than adults; in cats and humans, one-week-old neonates had about three to four times more REM sleep per 24 hours than adults. The high percent of neonatal REM sleep and its orderly decrease as the brain matures suggested to Roffwarg and later to Mirmiran and Corner and to Marks et al. that REM sleep had developmental functions. One strategy to expose the developmental functions of REM sleep was to deprive neonates of REM sleep and determine effects of REM sleep deprivation (RSD) on adult variables.

The neonatal RSD strategy was originally used in rats. Mirmiran and Corner studied the effects of pharmacological RSD of neonates on adult rat behaviors. Clomipramine or clonidine, each an efficacious REM suppressant, was administered daily for two weeks to neonatal rats. When the rats matured, compared with control rats, the treated rats had sexual deficiencies, REM sleep abnormalities, and changes in locomotor speed. Mirmiran and Corner interpreted their findings to indicate that neonatal REM sleep played important roles in the development of these behaviors.

Our lab suggested another interpretation of their findings. Our hypothesis was that in rats, neonatally administered clomipramine produced adult changes that modeled human endogenous depression. The hypothesis predicted that neonatally administered clomipramine produced a larger set of symptoms than studied by Mirmiran and Corner and that the larger set of symptoms resembled human endogenous depression. Subsequent studies on a wider range of possible depressive symptoms supported the hypothesis. Compared with control rats, adult rats treated neonatally with clomipramine (CLI rats) had several different behavioral, REM sleep, and physiological abnormalities found in human endogenous depression. Behavioral (sexual) abnormalities of CLI rats improved with treatment by the antidepressant drug imipramine. Studies by Hilakivi and Hilakivi also supported the interpretation that drug RSD of neonatal rats

Study Objectives: The present study describes a new method for instrumental REM sleep deprivation (RSD) of neonatal rats.

Design: In the new method, an experimental neonatal rat and a yoked control neonatal rat were singly housed in a small plexiglass chamber which was divided into two separate units by a vertical wall. The floor of the housing chamber was attached to the platform of a standard laboratory test tube shaker. EEG and EMG electrodes were implanted by the soft head plug method which permitted continuous, long-term polysomnography. EEG and EMG signals were sent to a computer that was programmed to turn on the shaker for 5 seconds whenever the experimental rat entered REM sleep.

Setting: NA

Patients: NA

Interventions: NA

Results: The shaking of the chamber usually terminated REM sleep by entry to slow-wave sleep or wake. Amount of RSD depended on the shaker's oscillation speed. At higher speed the method reduced REM sleep by more than 80%.

Conclusions: Thus, the new instrumental method of RSD can be used to study developmental functions of neonatal REM sleep. In particular, the instrumental method can test the hypothesis that in rats neonatal RSD produces the adult depressogenic effect of neonatally administered clomipramine.

Key words: Instrumental rem sleep deprivation; neonatal rats
produced depressive-like changes. In their studies, desipramine and zimeldine, antidepressant drugs that reduce REM sleep,20 when administered to neonatal rats, produced adult "behavioral despair" (lengthened immobility) in the Porsolt swim test.19 Also both drugs and nomifensin, another antidepressant drug that decreases REM sleep,20 administered to neonatal rats, increased adult alcohol consumption,21 a frequent symptom in depressed humans.

Thus four antidepressant drugs (clomipramine, desipramine, zimeldine, and nomifensin) and clonidine, administered to neonatal rats, produced adult symptoms resembling those found in human depression. The five drugs have different aminergic effects.22 Clomipramine preferentially but not selectively blocks NE uptake.22,23 Clonidine is an alpha-2 agonist.24 Nomifensin blocks both dopamine and 5HT uptake.22 On the other hand, each of the five drugs strongly suppresses REM sleep.20 Parsimony suggests that neonatal RSD by the drugs rather than their different aminergic effects mediated the adult depression.8 Consistent with this hypothesis, iprindole, an antidepressant drug that does not decrease REM sleep, did not produce adult depressive symptoms after administration to neonatal rats.25

These findings suggest the hypothesis that neonatal RSD mediates the depressogenic effects of neonatally administered antidepressant drugs.8 A test of this hypothesis requires a method in which effects of RSD are not confounded by effects of other variables. RSD by drugs is always confounded by the other, unavoidable effects of the drugs. Clearly, an unambiguous determination of the effects of neonatal RSD requires nondrug RSD. To accomplish this end Mirmiran and Corner tried the pendulum method of RSD.4 However, applied to neonatal rats, the method did not achieve the levels of RSD produced by the drugs. It also substantially decreased total sleep time, thus confounding the effects of RSD with the effects of total sleep deprivation. Marks, Roffwarg and colleagues used a variant of the small platform above water method to REM sleep deprive kittens.6 However, neonatal rats are too small (thumb-sized) for this method. In 1990, Shaffery et al. reported RSD of one kitten by a combination of vertical vibration of the kitten's cage at REM sleep onsets for 18 hours/day and total sleep deprivation by gentle experimental handling for six hours/day.26 The total sleep deprivation was done in four 1½ hour sessions for feeding. As a result of the vertical vibration, wake replaced REM sleep. This wake during RSD, in addition to the wake (total sleep deprivation) for feeding, reduced baseline daily total sleep time by about nine hours. Thus, effects of RSD were confounded by effects of sleep loss. Our search of the literature revealed no other method for instrumental RSD of neonatal rats.

The aims of the present work are (1) to describe a new instrumental method for continuous (24 hours/day), long-term (weeks) RSD of neonatal rats that avoids the confounds of pharmacological RSD and avoids confounds of sleep loss; and (2) to present preliminary data about the RSD efficacy of the new method. The new instrumental method of RSD was previously reported in an abstract.27

**METHOD**

The present work had prior approval of our Institutional Animal Care and Use Committee.

A brief description of the new method follows. Neonatal rats were individually housed in polysomnographic (PSG) recording chambers. At each computer detected REM sleep onset an instrument shook the rat's housing chamber for five seconds. The shaking usually terminated REM sleep and thus produced selective RSD. The apparatus provided access to food during spontaneous wakes between shaking episodes so interruptions of selective RSD by total sleep deprivation were not necessary for feeding. The apparatus, which oscillated horizontally rather than vertically, usually terminated REM sleep with slow-wave sleep rather than with wake. Consequently, RSD by the new apparatus was usually not confounded by sleep loss. Lastly, we developed a new electrode implantation procedure—the soft head plug method—which permitted continuous (24 hours/day), long-term (weeks) polysomnographic monitoring.28,29 As a result of combining the new shaking apparatus with the new electrode implantation, the method permitted uninterrupted, long-term selective RSD by mechanical rather than pharmacological means.

Three successive studies, which aimed at progressively larger RSD, were conducted. In these studies, 12– or 13-day-old Long-Evans hooded rats were implanted under metofane anesthesia with soft head plugs for PSG recordings. Continuous instrumental RSD was started the next day and continued for 48 hours (short-term RSD) or for nine days (long-term RSD).

The instrumental RSD method had three components: (1) a new method for long-term, continuous PSG recordings of neonatal rats;28,29 (2) a computer program that recognized sleep/wake states online and at each REM sleep onset turned on an apparatus that shook the neonatal rat cage for five seconds; and (3) the apparatus that shook the rat cage in response to the computer's signal.

<table>
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<th>Table 1.—REM sleep percent of 24 hours</th>
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<td><strong>Day 1</strong></td>
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(1) Long-term, Continuous PSG Recordings

Continuous (24 hours/day) PSG recordings of neonatal rats were obtained by the soft head plug technique described in the accompanying report.28,29 Briefly, an EEG electrode was a six inch length of thin but strong Teflon coated wire which 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. For electrical contact, about ¾ inch of the Teflon coat was removed from the end of the wire that contacted tissue. A tiny drop of tissue adhesive liquid was used to cover the skull. The same type of wire was implanted in the nuchal muscle for EMG recording. Physiological signals from the soft head plug were sent to a polygraph via a multichannel commutator and then through a data processor (Dap 3000/212 MicroStar Lab) to the computer. As described in the accompanying report,29 the soft head plug technique permitted continuous (24 hours/day), long-term (weeks) PSG recordings of neonatal rats. Unlike the conventional method in which a large, hard head plug was cemented to the skull, the soft head plug could be removed without injury or death of the neonates. Recorded neonates could then grow to maturity. Thus, the new PSG recording technique permitted study of the effects of neonatal RSD on adult variables, a study not possible with the conventional hard head plug cemented to the skulls of neonatal rats.

The temperature of the rat cage was maintained at 26-27 degrees C by a temperature controller and heating infra-red lights. A plastic plate (8½" x 6½" x 2") containing bedding was placed on the bottom of each housing unit of the cage.

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![Figure 1.—Effects of shaking stimulation on PSGs](image)

P15 and P21—postnatal ages 15 & 21 days; RS—REM sleep; SWS—slow-wave sleep; RSD—REM sleep deprivation; stimulation-shaker on
Fresh human infant formula (Enfamil®) was supplied in a plastic bowl (¾" high, 2" diameter). To prevent the milk from spilling during shaking, the bowl was put in a holder screwed to the plastic plate shaking floor.

(2) Online Computer Recognition of Sleep/wake States

Continuous (24 hours/day) polysomnograms (EEG and EMG) were obtained with simultaneous paper recordings and computer registrations. As mentioned above, PSG signals were sent to the computer via a data processor. Both data processing and the RSD apparatus were controlled by the software of DapView for Windows (MicroStar Lab, Bellevue WA). EEG and EMG amplitude were sampled at the rate of 64 Hz and stored in 30 second epochs.

The software displayed the average EEG amplitude and average EMG amplitude of each 30 second epoch in numeric mode. By online comparison of PSG recording with the display of 30 sec EEG and EMG amplitudes on the computer screen, the experimenter determined the EEG and EMG reference amplitudes for REM sleep, viz, low amplitude EEG and EMG. These reference amplitudes were used by the computer to turn on the shaker to terminate REM sleep. For this purpose the computer averaged EEG amplitude and EMG amplitude every two seconds (128 samples). When the averages were below the reference amplitudes for REM sleep, the computer signaled a five second ON command to the RSD apparatus (shaker) to terminate REM sleep. Figure 1 shows effects of shaking stimulation on PSG tracings. In there studies, the PSG recordings had to be closely watched for two reasons: (1) to be sure that the shaker started at the onset of REM sleep; (2) to make frequent adjustments of reference values because during the neonatal period EEG amplitude of each state increased daily.

(3) The Shaking Apparatus

An experimental neonatal rat (X rat) and a yoked control neonatal rat (YC rat) were individually housed in a small plexiglass chamber (15" long, 9½" wide, 13" high) which was divided into two housing units by a vertical wall. (Fig. 2) The floor of the housing chamber rested on and was fixed to the horizontal surface of a general laboratory shaker (8289 ID, Thomas Scientific), ordinarily used to shake test tube racks. At each REM sleep onset of the X rat, the computer turned on the shaker for five seconds. The shaking of the housing chamber usually terminated the RS episode by producing a transition to either wake or slow-wave sleep. Thus, X rats and YC rats experienced the same shaking. However, X rats were shaken only at REM sleep onsets while YC rats were shaken during the sleep/wake state that happened to occur at the time of the REM sleep onsets of the X rats. As a result, X rats were selectively deprived of REM sleep, while YC rats were not. When X rats were awake or in slow-wave sleep, YC rats had the opportunity for any sleep/wake state.

The shaker's oscillation speed was manually controlled by the position of a knob on the shaker. As described below, an increase in shaker speed increased RSD. Within each of the three studies, shaker speed was kept constant. To achieve progressively larger RSD across studies, shaker speed was increased from study 1 to study 2 and from study 2 to study 3. Shaker speed was 160-200 oscillations/min in study 1; 200-240 oscillations/min in study 2; and 240-300 oscillations/min in study 3. Each oscillation was an excursion of 2 cm.

In study 1, during cage shaking rats often slid across their cages. To remedy this problem in studies 2 and 3, five protruding screws were inserted in different areas of the floor of the X cage. The screws were obstacles to prevent rats from extended sliding during cage shaking. The screws did not injure the rats, probably because they only extended about 1 cm above a thick (1.5 cm deep) layer of soft bedding (bed-o'cobs, lab animal bedding, The Anderson, Maumee, Ohio).

In this paper RSD efficacy of experimental (X) rats is described by REM sleep reduction of X rats relative to control groups (i.e., relative to yoked controls treated with intermittent shaking and relative to maternally separated controls.) However, it is possible that either of the control conditions reduced REM sleep. If so, a measure of RSD efficacy relative to such a control would underestimate total RSD. Thus, the ideal way to describe RSD efficacy would be REM sleep reduction relative to baseline REM...
sleep without intermittent shaking and without maternal separation. Measurement of such baseline REM sleep would require PSG monitoring of unshaken neonates without maternal separation. However, at present, continuous PSG monitoring of neonatal rats is technically impossible without maternal separation. Over time, the rat mothers interfere with and/or remove the electrodes, wires, and head plugs. The best one can do is polysomnographically record neonatal rats for very brief sampling times (e.g., one hour) and assume that such brief maternal separation has little or no effect on REM sleep. With that assumption we compared REM sleep in Mirmiran's control neonatal rats (recorded for one hour intervals one to three times/day) with REM sleep in our home cage rats (recorded continuously 24 hours/day). Mirmiran's rats were with their mothers except for the one hour recording sessions. Our rats had continuous maternal separation in their home cages. In each group the recordings were made for eight consecutive days from age 14 days through age 21 days. In dose response terms, the study compared continuous eight day maternal separation with intermittent, very brief maternal separation for their effects on REM sleep. The results were that with one exception on each day—from age 14 days through age 21 days—mean REM sleep in continuously separated neonatal rats was not less than mean REM sleep in briefly separated rats. The results supported the hypothesis that the continuous maternal separation of our neonatal rats did not reduce REM sleep. Hofer's 1982 report also supported this hypothesis. He found that with periodic adequate milk feeding, maternal separation did not decrease REM sleep of neonatal rats. Thus, in the present paper, total RSD efficacy was measured by REM sleep reduction relative to maternally separated controls and, as such, probably represents total or "true" RSD.

RESULTS

Study 1

The aim of the first study was to determine the feasibility of long-term instrumental RSD of neonatal rats by the above described shaker method. A nine day duration of RSD was selected because beginning at age 14 days a similar duration of clomipramine administration to neonatal rats produced depressive symptoms when the rats matured.

The study involved twoX rats treated with RSD, twoYC rats, and five age matched maternally separated control rats (MSC rats) recorded in stationary cages in an earlier study. Electrodes were implanted at age 13 days. Instrumental RSD and PSG recording began at age 14 days, and continued for a total of nine consecutive days (through age 22 days).

At age 14 days, the rat pups appeared healthy, opened their eyes, and were fed by the experimenter at least four times with Enfamil formula and solid food. At age 15 days the pups actively sought food and were able to eat by themselves. Grooming, eating, and locomotor behaviors of X and YC rats were similar during the nine-day experiment and were grossly indistinguishable from MSC rats and from behaviors of neonatal rats raised in earlier studies with foster mothers.

Daily mean number of stimulations (5 second cage shaking episodes) were 2811±224, 1738±46, and 1185±331 (mean±SD) on deprivation days 2, 5, and 9 respectively. Thus, number of stimulations/24 hours decreased with rat age. This was consistent with the age decrease in REM sleep/24 hours in the MSC rats and the age decrease in number of REM sleep episodes in MSC rats over the age range studied.

The immediate behavioral response to stimulation by shaking was usually a postural change and movement. The PSG response to stimulation by shaking was usually an increase of EEG and/or EMG amplitude, producing a transition to slow wave sleep or awake. On some occasions the PSG recording after shaking did not show a stage change that indicated a termination of REM sleep. Mean percent of stimulations that resulted in termination of REM sleep were 63%±5, 65%±9, and 67%±9 (mean±SD) on RSD days 2, 5, and 9 respectively. Thus, the efficacy of REM sleep terminations by cage shaking did not change with age and with duration of the instrumental RSD.

PSG results on RSD days 2, 5, and 9 are shown in Figure 3.

Compared with maternally separated controls (MSC), the instrumental RSD reduced mean REM sleep by 42.2%±9.0, 41.4%±4.7, and 67.2%±0.6 on RSD days 2, 5, and 9.
respectively. Compared with YC rats, the instrumental RSD reduced mean REM sleep of X rats by 43.6±8.8, 34.8±5.3, and 34.2±1.3 on RSD days 2, 5, and 9 respectively. In both comparisons, on deprivation day 2, slow-wave sleep replaced the lost REM sleep, while on deprivation days 5 and 9, wake replaced the lost REM sleep.

PSG variables were about the same in YC rats and MSC rats, except that on deprivation day 9, MSC rats had more REM sleep (19.8%) than YC rats (11.4%). Thus, at the end of the nine days of instrumental RSD with relatively slow shaker speed, YC rats experienced RSD, though not as much as X rats.

Study 2

The aim of this study was to produce more RSD than achieved in study 1. To do so, two technical changes were made. The oscillation speed of the shaker was increased from 160–200 oscillations/minute in study 1 to 200–240 oscillations/minute in study 2 and five screws were implanted in the floor of the experimental cage. During shaking, the screws restricted the excursions of the X rats to shaking back and forth rather than longer sliding.

Two durations of RSD were studied at the faster shaking speed. The first pilot RSD procedure at a faster shaking speed was conducted for 33½ hours with one X rat and one MSC rat. When this proved feasible, a second RSD procedure at the faster speed was conducted for 48 hours on four additional X rats with 4 MSC rats. YC rats were not studied in these procedures. PSG implants were done at age 11 days (33½ hour procedure) or age 12 days (48 hour procedure), and RSD was begun the day after implantation.

In this study (#2), compared with MSC rats, X rats had 62.8±1.4 RSD on the first deprivation day and 67.6±4.0 RSD on the second deprivation day (mean±SD). In this study (#2) during RSD day 2, mean number of stimulations (cage shakings) per 24 hours was 1875. In the prior study, conducted at slower shaker speed during RSD day 2, mean number of stimulations per 24 hours was 2811. In this study (#2), 78% of stimulations terminated REM sleep, while in study 1 (slower shaker speed), 63% of stimulations terminated REM sleep. The changes from study 1 to study 2 also produced a larger RSD. On RSD day 2, X rats had 68% RSD in study 2 (faster shaker speed) and 43% RSD in study one (slower shaker speed). Thus, increasing shaker speed produced a greater RSD with fewer stimulations, each one of which was more likely to terminate REM sleep.

PSG results are shown in Figure 4. Compared with MSC condition, instrumental RSD had little effect on total sleep times, (i.e., most of instrumentally deprived REM sleep was replaced by slow-wave sleep).

Study 3

The aim of this experiment was to increase the RSD above the level reached in experiment 2. To do so, shaker oscillation speed was again increased from 200–240 oscillations/minute in study 2 to 240–300 oscillations/minute in study 3. Instrumental RSD was administered for 48 hours beginning at age 14 days. Two instrumental RSD rats and two YC rats were studied. PSG data of MSC rats from study 2 were used as a second control condition.

PSG results are shown in Figure 5. Compared with MSC, X rats had 85.2±0.9 RSD (mean±SD) on first deprivation day and 84.5±1.1RSD on second deprivation day. Compared with YC, X rats had 76.8±1.5 RSD the first deprivation day and 77.4±2.9 RSD on the second deprivation day. In study 2, which was conducted at a moderate shaker speed, compared with MSC rats, X rats had 67% RSD. Thus, just as increasing shaker speed from study 1 to study 2 increased RSD, increasing shaker speed further from study 2 to study 3 produced further increases in RSD. As shown in Figure 5, most of the instrumentally deprived REM sleep was replaced by slow-wave sleep. Total sleep time was about the same in X rats, YC rats, and MSC rats.

The number of rats per treatment group in each study (n=2 or n =4) was too small for meaningful statistical tests of group differences in REM sleep. However, combined over the three studies, X rats had significantly less REM sleep than MSC rats on day 1 (11% vs.35%)(p<0.001) and on day 2 (12% vs.30%)(p<0.001)(nonpaired t tests, two-tailed). Similarly, combined over the three studies, X rats had significantly less REM sleep than YC rats on day 1 (11% vs. 23%)(p<0.01) and on day 2 (12% vs. 34% (p<0.005). These results indicate that the new instrumental method produced significant RSD.
DISCUSSION

The nature of neonatal REM sleep, the target of the deprivation procedure in the present study, has recently become controversial. The conventional view that neonatal REM sleep is the same state as mature REM sleep has recently been challenged. It has been proposed that neonatal Active Sleep is not REM sleep but an undifferentiated state that developed into REM sleep and slow-wave sleep. In the challenging study, sleep/wake states of neonatal rats were scored behaviorally, not polysomnographically. Active Sleep epochs were identified by the presence of phasic muscle twitches during behavioral sleep. All epochs were subject to Fourier analysis. The study found that many epochs with high power in the delta (slow-wave) frequency range were behaviorally scored Active Sleep. This finding led to the conclusion that Active Sleep was not REM sleep but rather an undifferentiated state with REM sleep properties (phasic muscle twitches) and slow wave sleep properties (high delta wave power). Two findings in a recent study of ours on the ontogeny of REM sleep in rats were inconsistent with this challenge. First, in the neonatal rats, many epochs of polysomnographically scored slow-wave sleep had phasic muscle twitches. Had these epochs then undergone Fourier analysis, high delta power would have been found. Had these epochs been scored behaviorally, they would have been mistakenly identified as REM sleep. Thus, contrary to the challenge, epochs with high delta power, behaviorally scored as Active Sleep epochs, were probably slow-wave sleep, not REM sleep, and not an undifferentiated state with properties of REM sleep and slow-wave sleep. Note that the challenging study did not report the number and percent of behaviorally scored Active Sleep episodes with high delta power. Our prediction is that in these converse data, the percent of Active Sleep episodes with high delta power will be low. Second, the study found that REM sleep lost during development was mainly replaced by wake, not by slow wave sleep as predicted by the hypothesis that early neonatal REM sleep is an undifferentiated state that developed into REM sleep and slow-wave sleep. These considerations suggest that polygraphic REM sleep in neonatal rats is not an undifferentiated REM sleep slow-wave sleep state but rather is the same state as REM sleep in mature rats. Thus, the target of the shaking stimulation in the present study was REM sleep and the result was RSD.

The main finding in the present study was that, compared with control rats, large, continuous RSD of X neonatal rats was achieved for several days by the new instrumental method. Effects on experimental rats of shaking and maternal separation together were controlled by study of yoked control rats. Effects on experimental rats of only maternal separation were controlled by study of maternally separated rats in stationary cages. In terms of gross appearance, behavior, and maturational signs, the method appeared harmless. Thus, the method can be used for long-term, continuous RSD of very young neonatal rats beginning five or six days before the usual age of weaning (age 17 or 18 days). An anonymous reviewer suggested that the efficacy of the shaking platform method in producing RSD of neonatal rats raises the possibility that the method might work for RSD of adult rats and would avoid confounds of the flower pot method (e.g., wetness and confinement).

Amount of RSD by the instrumental shaking method varied directly with the shaker's oscillation speed. Increasing the speed of the shaker's oscillation increased RSD with fewer stimulations and with a smaller loss of total sleep than at slower shaking speeds. The explanation of this finding is unknown. One speculation is that compared with slower shaking speed, higher shaking speed terminated REM sleep more quickly and thereby was less arousing.

Long-term instrumental RSD (nine consecutive days) was conducted only at the slower shaking speed. The reduction of REM sleep by the instrumental method was about the same at the start and end of the nine days. The finding indicates that the instrumental method did not lose its RSD efficacy over the nine day period. At the end of the nine day procedure, REM sleep was replaced by wake, not by slow-wave sleep as occurred at the start of the procedure. The present study did not determine whether the loss of sleep at the end of the process was a result of slow shaker speed or of the duration of RSD.

Compared to saline control treatment, clomipramine administered to neonatal rats at depressogenic doses, reduced neonatal REM sleep by 77% (Our calculations from a published graph). This result was based on daily one to three hour samples of PSG recordings obtained sequentially from different rats. In our lab, a recent, as yet
unpublished study of neonatal rats recorded 24 hours per day, found that clomipramine in depressogenic doses reduced daily REM sleep by 56% over a similar period. The present study found that compared to maternally separated controls, instrumental RSD produced 84% RSD at the highest oscillation speed and 68% RSD at the moderate oscillation speed. Thus, the level of RSD produced instrumentally by higher shaker speeds exceeded the RSD levels produced pharmacologically by depressogenic clomipramine and the RSD level of moderate shaking speed exceeded the lower estimate of RSD required for depressogenic effects. Also, there was no evidence in the present study that at a given oscillation speed, the instrumental RSD decreased over successive RSD days, even over the nine days of treatment at the lowest oscillation speed.

In conclusion, the present findings suggest that instrumental RSD by the shaker can produce a large RSD for a relatively long period. This means that the instrumental RSD method can be used to study developmental functions of neonatal REM sleep without the confounds introduced by the drug method of RSD. In particular, the instrumental method can be used to test the hypothesis that neonatal RSD mediates the depressogenic effects of neonatally administered clomipramine and to investigate possible paradoxical effects of RSD which depend on age (i.e., RSD of adults improves depression and RSD of neonates causes depression).

REFERENCES

31. Hofer MA, Shair H. Control of sleep-wake states in the infant rat.

32 Unpublished data in our laboratory. 1999.


