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Dose-Dependent Decrements in Adult Male Rat Sexual Behavior After Neonatal Clorimipramine Treatment

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VOGEL, G., M. HAGLER, A. HENNESSEY AND C. RICHARD. *Dose-dependent decrements in adult male rat sexual behavior after neonatal clorimipramine treatment.* PHARMACOL BIOCHEM BEHAV **54**(3) 605–609, 1996.—Previous studies found that months after clorimipramine (CLI) treatment of male neonatal rats, the mature animals developed behavioral deficits and REM sleep abnormalities that modeled human endogenous depression. In the initial studies neonatal rats received CLI 30 mg/kg/IP daily from age 8 through 21 days. Diminished sexual activity of the adult rats treated neonatally in this manner was a behavioral deficit that supported the depression model. However, in subsequent studies in our laboratory, the same neonatal treatment occasionally failed to produce adult sexual deficiencies found in the initial studies. The inconsistency raised the possibility that neonatal CLI treatment was not a reliable method to produce an animal model of depression. An alternative hypothesis was that the CLI dose was too low. The present study tested this hypothesis. Placebo or one of four CLI doses was administered daily to male neonatal rats (n = 12/treatment group) from age 8 days through 21 days: 30 (the original dose), 40, 50, and 60 mg/kg/IP. Six components of adult sexual behavior were measured at age 5 months. Deficiency of each sexual behavior was found to be dose-dependent (r = 0.5, $p < 0.001$). The 30 mg/kg/day dose caused deficiencies in some, but not all, sexual behavior measures. Higher doses caused deficiencies in all measures of sexual behavior. The results support the hypothesis that neonatal CLI treatment at doses higher than the original 30 mg/kg/day caused reliable impairments in adult male rat sexual behavior, and thereby support the reliability of neonatal CLI treatment to produce an animal model of endogenous depression.

Animal depression model Male rat sexual behavior Neonatal clorimipramine Neonatal antidepressant drugs

CLORIMIPRAMINE treatment of neonatal rats (CLI rats) resulted in later, adult abnormalities that modeled human endogenous depression (15,20). Compared with adult control rats that had received saline as neonates (SAL rats), the adult CLI rats had behavior abnormalities found in human endogenous depression: decreased sexual (10), aggressive (14), pleasure-seeking (intracranial self-stimulation) activities (16); and REM sleep abnormalities (17) found in human endogenous depression (1,2,6,18,21): increased REM sleep, decreased REM latency, increased number of sleep onset REM periods, abnormal temporal course of REM rebound with a normal total REM rebound. In preliminary studies the behavioral abnormalities were improved by treatments that improved human endogenous depression: imipramine and REM sleep deprivation by arousal (15). Also, the course of the rat disorder (15) resembled the course of human endogenous depression: spontaneous adult development without a stressful precipitant, autonomous course lasting months; a possible spontaneous improvement months after onset. In other support of the depression model, adult CLI rats had the depressive response in the forced swim test (13). These abnormalities in CLI rats suggested a wide range of features similar to those found in human endogenous depression and, therefore, supported the validity of the animal model.

Although other investigators recently reported that treatment of neonatal rats with CLI produced sexual deficiency in adult rats (12), more recent unpublished work in our laboratory found that the usual neonatal treatment of CLI (30 mg/kg/day from age 8 days to 21 days) did not consistently produce the adult sexual deficiencies found in earlier studies.

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The inconsistency suggested that neonatal CLI treatment might not be a reliable method of producing an animal model of endogenous depression. An alternative hypothesis was that the inconsistency occurred because the CLI dose (30 mg/kg/day) was too low. According to the dose-dependent hypothesis, neonatal CLI produced a dose-dependent decrease in sexual behavior so that sexual deficiencies would be inconsistently found at the 30 mg dose and more consistently found at higher doses. The present study tested this hypothesis. Male rats were neonatally treated with one of five CLI doses (0, 30, 40, 50, and 60 mg/kg/day) from age 8 days through 21 days and their sexual behavior was tested at age 5 months.

METHOD

Animals

Twenty-three timed pregnant Long-Evans rats produced 133 male rat pups. The rats were crossfostered and treated as previously described (5,10,14,16,17). In brief, from age 8 days through 21 days the 133 male rat pups were treated daily with one IP injection clomipramine. Each rat received one of five doses of clomipramine: 0 mg/kg (n = 27); 30 mg/kg (n = 27); 40 mg/kg (n = 27); 50 mg/kg (n = 26); and 60 mg/kg (n = 26). The 0 mg/kg group received equivolume saline. The colony room was on a reversed 12 L : 12 D cycle with lights on at 1330 h. Food and water were available ad lib.

Within 2 weeks of treatment 14 rats died and 19 other rats were sacrificed because they developed diarrhea and abdominal distensions, prodromal symptoms of the deaths. Autopsy of two dead rats in the 60 mg group revealed both had injections of CLI into the large intestine, in one causing adynamic ileus and in the other causing peritonitis. Losses in each treatment group were: 1 rat in the saline group, 2 rats in the 30 mg group, 6 rats in the 40 mg group, 10 rats in the 50 mg group, and 14 rats in the 60 mg group. Thus, of the original 133 rats, 100 survived the treatment, appeared well, were weaned at age 1 month, and housed in individual cages. These 100 rats included 26 rats in the saline group, 25 rats in the 30 mg group, 21 rats in the 40 mg group, 16 rats in the 50 mg group, and 12 rats in the 60 mg group.

At age 4 months the 100 rats were tested for locomotion in an open-field apparatus. Sixty of these rats were then selected for testing of their sexual behavior, and the remaining 40 rats were used for other studies. The 60 rats were chosen as follows. The smallest treatment group was the 60 mg group, which had 12 animals. We, therefore, decided to test sexual activity of 12 rats per treatment group, and chose, by random selection, 12 rats from each of the other treatment group. Sexual testing was begun at age 5 months. During the interim between open-field and sexual testing, one rat in the 60 mg group died of undetermined causes. Thus, the final testing of sexual activity was done on 11 rats in the 60 mg group and 12 rats in each of the other groups.

Behavioral Testing

When the rats were 4 months of age, the first behavioral test was conducted to assess locomotor activity. Animals were tested daily for 2 min in an open-field apparatus on 5 consecutive days. As previously described (5), open-field testing was conducted in a circular chamber measuring 70 cm internal diameter with 30 cm high walls. Floor and walls were lined in white formica; the floor was marked into three concentric rings transected by lines arranged like spokes on a wheel, forming pie slice-shaped sectors. The apparatus was placed on the floor in a quiet room with a 75 W white incandescent bulb suspended 90 cm over the center of the open field. Behavioral testing was conducted during the dark phase of the lighting cycle. At the start of each 2-min test session the animal was placed in the center of the field. Sectors entered by each rat were recorded by an experimenter, blind to the treatment of the test animal. The floor was sanitized between test sessions.

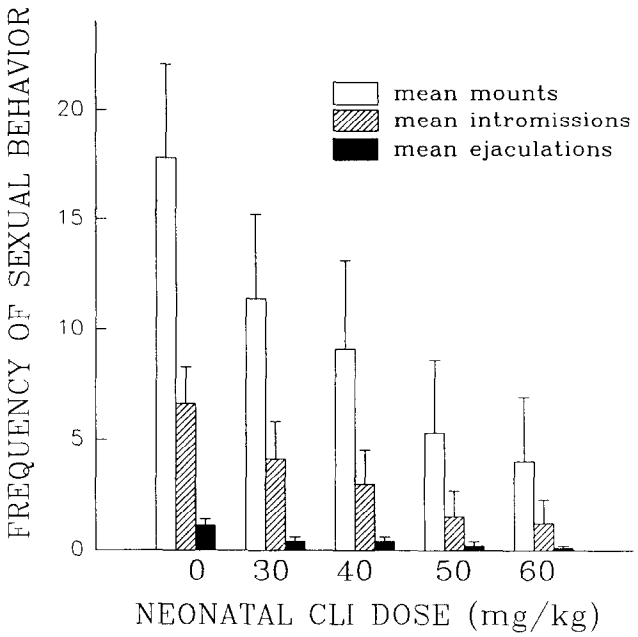

FIG. 1. Effect of neonatal clorimipramine dose on three adult male sexual behaviors, each displayed as mean + standard error of the mean (SEM).

When the rats were 5 months of age, each male rat was tested for sexual behavior on three separate occasions with 2 days between each test. The tests used a standard paradigm (3), which was used in our previous studies of rat sexual behavior (5). Briefly, rats were sexually naive at the start of testing. Before introducing a stimulus 5 month old female, the male was placed in a cylindrical Plexiglas observation chamber (2 ft high with a diameter of 1 ft) for a 10-min adaptation period. The testing chamber was illuminated by a dim (25 W) red bulb. The test was initiated by placing a sexually receptive female into the arena with the male. Females were brought into heat by injections of estrogen (estradiol benzoate, 5 μg injected SC in 0.05 ml corn oil, 48 and 24 h before testing) and progesterone (500 μg injected SC in 0.1 ml oil 4–6 h before testing). On a given day each female was paired with only one male and on different days male-female partners were changed. The order of testing of male rats was randomly determined. Each test lasted 30 min and the test was conducted during the dark phase of the light : dark cycle. Wood shavings on the floor of each chamber were changed after the completion of each test. The technician observing sexual behavior was blind to neonatal treatment and was reliable in the scoring of rat sexual activities.

Sexual Behavior Measures

The following indicators of male sexual behavior were measured: number of mounts, number of intromissions, num-

ber of ejaculations, latency (time) to first mount, latency (time) to first ejaculation, and postejaculatory interval (mean time from ejaculation to next mount).

Statistical Analysis

Statistical differences among treatments were analyzed using a two-way (treatment and day) analysis of variance (ANOVA) with a repeated measures design for days. Post hoc pair-wise comparisons of treatment groups were evaluated by the Bonferroni test. Correlations were determined by the Pearson Product-Moment test. Significance was ascribed at $p < 0.05$ (Crunch Software Corp., V. 3.0, Oakland, CA).

RESULTS

At age 4 months, the five treatment groups of 100 rats were not significantly different in any measure of open-field locomotion. Also the five treatment groups of 60 rats later selected for sexual testing were not significantly different in any measure of open-field locomotion. Specifically, the five treatment groups of 60 rats were not significantly different in total number of sectors entered; in number of outer, middle, or inner sectors entered; and in time spent in outer, middle, or inner sectors (all $p > 0.35$). Nevertheless, treatment groups differed significantly in number of fecal boli during open-field testing ($p < 0.009$). In post hoc, pair-wise comparisons of treatments groups, all CLI treated groups had more fecal boli than SAL rats (pair-wise p range from 0.095 to 0.012, Bonferroni) but there was no significant difference between any two CLI treatment groups (all $p > 0.9$).

The effects of neonatal CLI treatment on adult sexual activity (mounts, intromissions, and ejaculations) are illustrated in Fig. 1. As CLI dose increased, frequency of each sexual activity decreased. The correlation between dose and each sexual activity was about -0.5 ($p < 0.001$) (see Table 1). Thus, neonatal CLI treatment produced a dose-dependent decrease of male adult sexual activity.

The five treatment groups were significantly different in frequency of mounts, $F(4, 58) = 5.5$, $p < 0.001$, intromissions, $F(4, 58) = 5.1$, $p < 0.001$, and ejaculations, $F(4, 58) = 8.3$, $p < 0.001$ (Table 1). In pair-wise comparisons, compared to SAL animals, CLI animals treated with 30 mg/kg had significantly fewer ejaculations ($p < 0.01$, Bonferroni), but their reductions in mounts and intromissions were not statistically significant. Compared to SAL rats, CLI rats treated with 40 mg/kg also had significant reductions in ejaculations, and their reductions in mounts and intromissions were not quite significant ($p < 0.10$). Finally compared to SAL rats, animals that received the highest doses of CLI (50 mg/kg and 60 mg/kg) had significantly fewer mounts ($p < 0.01$), intromissions ($p < 0.01$) and ejaculations ($p < 0.001$) than SAL animals (Bonferroni) (Table 1).

Across all rats, each sexual behavior was significantly different on the three test days: mounts, $F(2, 118) = 21.4$, $p < 0.001$, intromissions, $F(2, 118) = 20.8$, $p < 0.001$, and ejaculations, $F(2, 118) = 16.8$, $p < 0.001$. Changes in sexual activity over the 3 test days were similar for each behavior. Sexual behavior decreased or was unchanged from the first to the second test day and then rose to a 3-day maximum on the third test day. Over the 3 test days all treatment groups had similar changes in mounts and intromissions (no significant treatment by day interaction). Ejaculations showed a significant interaction of test day and treatment, $F(2, 118) = 2.2$, $p < 0.05$. Animals treated with the two highest doses of CLI ejaculated only on test day 3.

Like sexual activities, sexual latencies (mount latency, ejaculation latency, and postejaculatory pause) were significantly affected by treatment (Fig. 2). As CLI dose increased, each latency measure increased. The correlation between neonatal CLI dose and each sexual latency was about 0.5 ($p < 0.001$) (Table 1). Thus, neonatal CLI produced a dose-related increase of sexual latencies.

The five treatment groups were significantly different in latency: latency to mount, $F(4, 58) = 5.3$, $p < 0.001$, latency to ejaculate, $F(4, 58) = 8.5$, $p < 0.0001$, and postejaculatory interval, $F(4, 58) = 6.9$, $p < 0.0001$ (Table 1). In pair-wise

TABLE 1

DOSE RELATED EFFECTS OF NEONATAL CLI ON SEXUAL BEHAVIORS

	Neonatal CLI Dose (mg)					Differences Among All Treatment Groups p	Dose Response r (p)
	0	30	40	50	60		
Number of mounts	17.8 ± 4.2	11.4 ± 3.8	9.1 ± 4.0 Δ	5.3 ± 3.3 **	4.0 ± 2.9 ***	***	−0.52 (***)
Number of intromissions	6.6 ± 1.7	4.1 ± 1.7	3.0 ± 1.5 Δ	1.5 ± 1.2 **	1.2 ± 1.1 **	**	−0.51 (***)
Number of ejaculations	1.1 ± 0.3	0.4 ± 0.2 **	0.4 ± 0.2 **	0.2 ± 0.2 ***	0.1 ± 0.1 ***	***	−0.55 (***)
Mount latency	460 ± 94	939 ± 143	1203 ± 194 *	1199 ± 151 *	1370 ± 178 **	**	0.49 (***)
Ejaculation latency	1135 ± 97	1544 ± 81 **	1550 ± 104 **	1681 ± 55 ***	1759 ± 41 **	***	0.56 (***)
Postejaculation latency	1052 ± 111	1428 ± 124	1468 ± 183 *	1638 ± 69 **	1753 ± 47.2 **	***	0.55 (***)

Pairwise comparisons of the treatment groups were evaluated by the Bonferroni test. A significant difference in sexual effect between saline (0 mg CLI) and a CLI dose is indicated by asterisks or delta immediately below the entry in the CLI dose column. There were no significant differences between 30 mg CLI and a higher dose or between 40 mg CLI and a higher dose. Each entry is the mean (±SEM) of all rats in a given treatment group.

***$p \leq 0.001$; **$p \leq 0.01$; *$p \leq 0.05$; Δ$p \leq 0.1$.

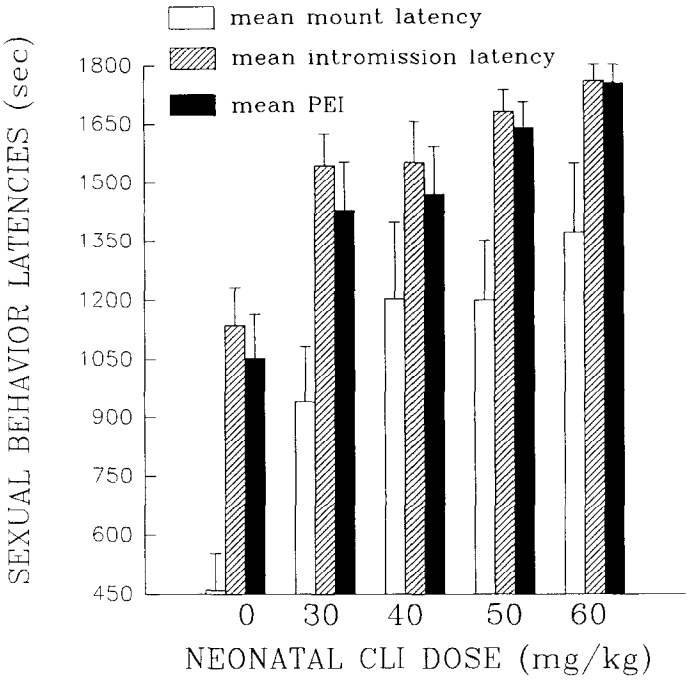

FIG. 2. Effect of neonatal clorimipramine dose on three adult male sexual latencies, each displayed as mean + standard error of the mean (SEM).

comparisons (Bonferroni), compared with SAL rats, CLI rats that had received 40, 50, or 60 mg/kg had significantly longer mount latencies, ejaculation latencies, and postejaculatory pauses. CLI rats that had received 30 mg/kg (lowest dose) had significantly longer ejaculation latencies than SAL rats but differences between 30 mg CLI rats and SAL rats in mount latency and postejaculatory latency did not reach statistical significance (Table 1).

Test day had a significant effect on mount latency, $F(2, 118) = 16.6$, $p < 0.0001$, latency to ejaculate, $F(2, 118) = 16.4$, $p < 0.0001$, and postejaculatory interval, $F(2, 118) = 12.8$, $p < 0.0001$. The direction of these effects was similar for each measure. CLI-treated animals had the shortest latencies on the third test day. Over the 3 test days all treatment groups had similar changes in mount latencies and postejaculatory interval (no significant treatment by day interaction). A significant interaction between test day and treatment dose was found for ejaculation latency, $F(2, 118) = 2.1$, $p < 0.05$.

DISCUSSION

The present study found that neonatal clorimipramine treatment caused dose-dependent impairments of all six measured sexual behaviors of adult male rats. Specifically, neonatal treatment with clorimipramine caused significant dose-dependent decreases in the frequency of mounts, intromissions, and ejaculations (Fig. 1), and significant dose-dependent increases in latency to mount, latency to ejaculate, and postejaculatory interval (Fig. 2). The six correlations between neonatal CLI dose and sexual variables were approximately the same (about ± 0.50) ($p < 0.001$). Thus, neonatal CLI treatment had about equal effects on each of six measured aspects of adult sexual behavior. The findings indicate that neonatal CLI treatment produced a dose-dependent global sexual deficiency in adult CLI rats.

Previous work in our laboratory (5) and in other laboratories (7,8) found that neonatally administered CLI usually, but not always (4,9), produced adult rats that were hyperactive in an open-field apparatus. In the present study, neonatally administered CLI had no effect on locomotor activity in an open field. We have no explanation for these inconsistencies. Nevertheless, because the present study found no significant difference among treatment groups in adult locomotor activity, sexual differences among treatment groups could not have been a result of motoric differences among treatment groups—at least as they were measured by open-field activity. In particular, the CLI dose-related decrease in sexual activity could not have been a result of a decreased motoric activity because this study did not find that neonatal CLI decreased adult motoric activity. The study did find that compared with SAL rats, all CLI treatment groups had more fecal boli—although the CLI treatment groups did not differ from one another. The finding suggests that neonatal CLI treatment increases the emotionality or anxiety of mature rats in a nondose-related fashion. Because anxiety is a regular component of human endogenous depression, the finding that CLI rats had an indicator of anxiety is consistent with these animals being a model of endogenous depression. The finding that boli were not CLI dose related—as were sexual deficiencies—indicates that sexual deficiencies cannot easily be explained as the result of emotionality or anxiety.

About one-quarter of the neonates that received CLI developed diarrhea and/or abdominal distension—and about 10% of rats died within 2 weeks of treatment. Higher CLI doses were more likely to produce the illness. Autopsy suggested that the symptoms occurred because IP CLI, particularly in high doses, irritated the peritoneal cavity producing peritonitis, or was inadvertently injected into the colon producing adynamic ileus and abdominal distension. There was no gross evidence that the 75% of the animals without the abdominal

symptoms were sick or failed to develop normally. This is supported by the finding that at age 4 months the CLI treatment groups were not significantly different from SAL rats in open-field locomotion.

In conclusion, the present findings were that, administered to neonates, the 30 mg/kg/day dose of CLI had inconsistent effects on the six measured adult sexual variables. Some variables were significantly decreased and others were not. Thus, the present findings replicate earlier findings that, administered during the neonatal period, 30 mg/kg/day of clorimipramine had inconsistent effects on adult sexual behavior. CLI doses of 40 mg/kg affected all six measured sexual variables, producing either a trend toward significant impairment or a significant impairment. CLI doses of 50 mg/kg and 60 mg/kg significantly impaired all six measured sexual variables. Based on these results, we suggest a neonatal dose of clorimipramine 40 mg/kg/day will reliably produce adult depression-like impairments in large *n* experiments (20 rats/group) and a neonatal dose of 50 mg/kg/day will reliably produce adult impairments in smaller *n* experiments. In general the present results support the hypothesis that, at doses higher than the original 30 mg/kg dose, CLI treatment of neonatal rats is a reliable method for producing adult rat abnormalities that model human endogenous depression.

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