Pharmacology Biochemistry & Behavior, Vol. 31, pp. 103–106. © Pergamon Press plc, 1988. Printed in the U.S.A. 0091-3057/88 $3.00 + .00

Animal Depression Model by Neonatal Clomipramine: Reduction of Shock Induced Aggression

G. VOGEL,[1] P. HARTLEY,* D. NEILL,* M. HAGLER AND D. KORS

Sleep Laboratory, Department of Psychiatry, Emory University School of Medicine and the Georgia Mental Health Institute, Atlanta, GA 30306
**Department of Psychology, Emory University, Atlanta, GA 30322*

Received 7 December 1987

VOGEL, G., P. HARTLEY, D. NEILL, M. HAGLER AND D. KORS. *Animal depression model by neonatal clomipramine: Reduction of shock induced aggression.* PHARMACOL BIOCHEM BEHAV 31(1) 103–106, 1988.— Clomipramine, administered to neonatal rats, has been reported to produce adult behavioral and REM sleep abnormalities. They include decreased sexual behavior, increased ambulation in the outer part of an open-field arena, increased REM sleep % of total sleep time, and in descriptive data, short REM latency, and increased REM phasic events. Since these abnormalities resemble some found in human endogenous depression, we have hypothesized that the adult rats represent an animal model of depression. Diminished aggressive behavior is a common characteristic of endogenous depression. This study tested the validity of the animal depression model by determining in rats the effect of neonatal clomipramine on adult shock-induced fighting. Experimental rats were treated neonatally with clomipramine and control rats were treated neonatally with saline. When they matured, compared with control rats, experimental rats had significantly fewer offensive fighting responses, and significantly more defensive fighting responses. The findings add some support to the validity of the animal depression model produced by neonatal clomipramine.

Animal depression model Rat aggression Neonatal clomipramine Neonatal antidepressant drugs REM sleep Shock-induced fighting

TWO independent approaches have led to the hypothesis that in rats neonatally administered antidepressant drugs produce adult animals that model human endogenous depression. The first approach emphasized the effect of neonatal clomipramine on REM sleep (14). The drug produced neonatal REM sleep deprivation which was followed by a lifelong increase of REM sleep (9,10). When mature, the rats showed several abnormalities analogous to some frequently seen in humans with endogenous depression (14). These included impaired sexual activity, increased ambulation in peripheral areas of an open field but not in central areas, and in nonsystematic pilot data, short REM latency and increased phasic events during REM sleep (9,10). Consistent with findings that REM sleep deprivation improved human endogenous depression (15) and augmented motivated animal behaviors (13) we hypothesized that the permanent increase of REM sleep, or some correlate of it, produced the adult rat behavioral decrements (14). The second approach to the animal depression model emphasized the effects of neonatal desipramine or zimeldine on forebrain monoamines (4). The neonatal antidepressant drugs produced adult animals with alterations in forebrain monoamines, increased alcohol consumption, and 'behavioral despair' as measured by the Porsolt swim test (4–6).

Clinical experience and a factor analytic study of symptoms in depressed patients indicated that aggressive behavior was decreased in human endogenous depression (12). This suggests that animals modelling endogenous depression will show less aggressive behavior than control animals. The present study tested this prediction of the animal depression model produced by neonatal clomipramine.

METHOD

Pregnant female Sprague-Dawley derived rats were obtained from Harlan/Sprague-Dawley (Indianapolis, IN). These rats whelped one week after arrival in our laboratory. Three days postnatally, all male pups were cross-fostered and all female pups were killed. Cross-fostering consisted of removing all pups from their biological mothers and placing them with another lactating female (the nonbiological foster mother). In the process each litter was divided into two groups of approximately equal number. Each half of the litter was placed with a different lactating female (the foster

[1]Requests for reprints should be addressed to Gerald W. Vogel, M.D., Emory Sleep Laboratory, Georgia Mental Health Institute, 1256 Briarcliff Road, N.E., Atlanta, GA 30306.

TABLE 1
MEAN NUMBER OF INDIVIDUAL BEHAVIORS PER ANIMAL

	Offensive				Defensive				Mutual Upright		
	Day 1	Day 2	Day 3		Day 1	Day 2	Day 3		Day 1	Day 2	Day 3
Control (C)											
Upright	19.6	15.8	14.9	Supine	0.7	2.0	1.5	Control	5.4	12.2	12.9
Crouch	0.4	0.6	0.0	Upright	2.5	2.7	4.2	Experimental	5.7	11.3	12.7
Leap	0.3	0.5	1.5	Crouch	3.4	1.9	1.8				
Mount	0.4	0.1	0.8								
Total	20.7	17.0	17.2	Total	6.6	6.6	7.5				
Experimental (X)											
Upright	6.5	6.6	6.0	Supine	3.0	3.2	2.0				
Crouch	0.3	0.5	0.0	Upright	8.5	7.3	9.0				
Leap	0.4	0.0	0.9	Crouch	8.1	5.6	4.9				
Mount	0.1	0.1	0.4								
Total	7.3	7.2	7.3	Total	19.6	16.1	15.9				

mother), and each foster mother received half the pups from two litters. All pups with each foster mother received the same treatment (clomipramine or saline) and the two pup groups of each original litter were assigned to different treatments. Thus, each original pup litter contributed equally to experimental (X) and control (C) groups. Each pup was injected subcutaneously between the shoulder blades with clomipramine hydrochloride (Anafranil, Ciba-Geigy Corp.) 15 mg/kg (3 mg/ml) or equivolume isotonic saline vehicle twice daily (0800 and 1800 hr) on postnatal days 8 through 21. At one month of age the pups were weaned and housed as litters until approximately 3 weeks prior to testing, at which time they were individually housed.

All testing was conducted in a Coulbourn operant conditioning chamber (Lehigh Valley, PA). The chamber was constructed of stainless steel and Plexiglas with 8 mm dia. stainless steel rod floors. The floor rods were connected to a BRS/LVE shock generator, model SG-903 and shock scrambler, model SC-922. The chamber was placed in a quiet room separate from other testing areas in the laboratory.

Behavioral testing commenced at 3 months postnatally. Each test pair consisted of one drug-treated and one vehicle-treated rat. Prior to testing, all drug- and saline-treated rats were paired by body weight (within 5 grams) to diminish size differences that could affect the behavioral results. Ten pairs of rats were tested. Pairs remained the same throughout testing.

Tests were done daily for 4 days. On the first day, animal pairs were placed in the chamber for 12 min habituation. On days 2–4 (called test days 1–3), the sessions started with a 2 min habituation period followed by 10 min of shock delivery. Shocks (1.33 mA, 0.5 sec duration) were delivered on a variable schedule with a minimum of 5 and maximum of 10 sec intershock interval. This resulted in a total of 70–80 shocks within the 10-min session.

Two observers scored simultaneously. Each watched one rat of the pair. The rats were identified by a red mark placed on the fur of one. The behaviors produced were almost totally in response to shock delivery; both animals tended to be immobile between shocks. Both observers were blind to the treatment condition of the animals. Prior testing showed an interobserver reliability of 0.99.

Offensive and defensive behaviors were scored using an observational system which we devised based on a description of aggressive behavioral topography for rats (8). Dominant and submissive behaviors were clearly distinguishable. Offensive or dominant behaviors included the following 4 behaviors. 1) An upright offensive posture was part of a mutual-upright posture in which the dominant rat towered over the submissive rat. The submissive rat reared on its hindfeet, with the head positioned at an upward angle, the forepaws extended toward the attacking animal, and the ventral surface of the body continually facing the opponent. 2) In the offensive crouch, the dominant animal turned its flank towards the subordinate; the submissive crouch was characterized by freezing in a motionless crouching position. 3) Mounting behavior was frequently seen and was the same as that seen in the male prior to copulating with a female. 4) Leaps in response to the shock which were directed toward the other rat were scored as aggressive responses. Three defensive or submissive behaviors were observed: 1) defensive upright posture, 2) submissive crouch (both described above), and 3) a supine position in submission to the dominant rats. Note that in this system each offensive behavior by one rat was partly defined by a defensive behavior of the other rat and vice versa. Thus, if the two independent raters of rat behavior were reliable, total offensive behaviors of one rat group should approximately equal total defensive behaviors of the other rat group and total fighting activity of the two groups (sum of offensive and defensive behaviors) should be the same.

If the rat was leaping in response to the shock but not directing the response toward the other rat, or both rats were in a mutual upright posture which could not be scored as defensive or offensive, no score was given.

RESULTS

Table 1 lists the individual behaviors of the two treatment groups on each test day. Plots of mean total offensive and defensive behaviors by each treatment group on the three test days are shown in Figs. 1–3. A 2×3 (treatment × days) analysis of variance for repeated measures was applied to offensive, defensive, difference (offensive minus defensive)

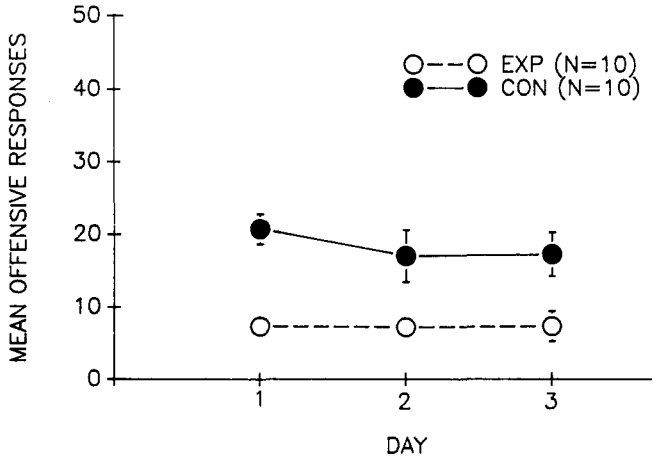

FIG. 1. Mean offensive fighting responses ±SEM.

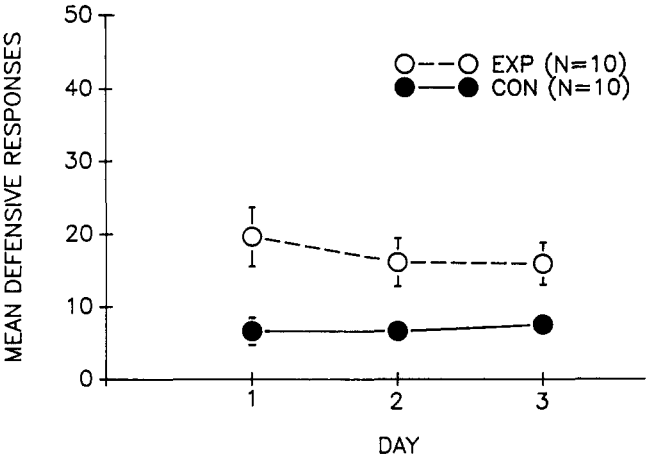

FIG. 2. Mean defensive fighting responses ±SEM.

and total (offensive plus defensive) behavior scores. Compared with C rats, X rats had significantly fewer offensive behaviors, $F(1,54)=23.75$, $p<0.001$, significantly more defensive behaviors, $F(1,54)=23.79$, $p<0.001$, and a significantly larger difference between offensive and defensive behaviors, $F(1,54)=35.48$, $p<0.001$. There were no significant day effects or treatment × day interactions. Total fighting activity (sum of offensive plus defensive behaviors) was not significantly different in X and C rats, $F(1,54)=0.19$, $p>0.05$.

DISCUSSION

The findings indicated that neonatal clomipramine diminished aggressive behavior in mature rats. X and C animals were not significantly different in sum of aggressive and defensive activities. As explained in the Method section, this indicated that the independent ratings of the two testers were reliable. Could X-C differences in shock-induced aggression have been a result of X-C differences in pain threshold? Only indirect evidence is available on this question. In one study REM sleep deprivation decreased pain threshold measured by tail arch in restrained rats (2). However, the flinch jump test measures pain threshold under conditions that more closely resemble conditions of the present study than those in the tail arch test on restrained rats. Measured by the flinch jump test, REM sleep deprivation increased pain threshold (3). Since C animals probably had less REM sleep than X animals, C animals, like REM-deprived animals, may have had a higher pain threshold than X animals. If so, X-C pain threshold differences cannot explain X-C aggression differences.

The present finding of diminished aggression in X animals may be added to the prior evidence that X animals show altered sexual and exploratory behaviors; and may show short REM latency and increased REM phasic events. The combination of findings supports the validity of the animal model of depression. Nevertheless, additional studies are necessary to test the validity of the animal model. As further depression indicators, we have suggested that, like humans with endogenous depression, the "depressed" animals should show 1) behavioral indications of anhedonia; 2) an abnormal temporal course of REM rebound after REM sleep deprivation; and 3) behavioral "improvement" with antidepressant treatments (14).

The pathogenesis of the adult behavioral abnormalities

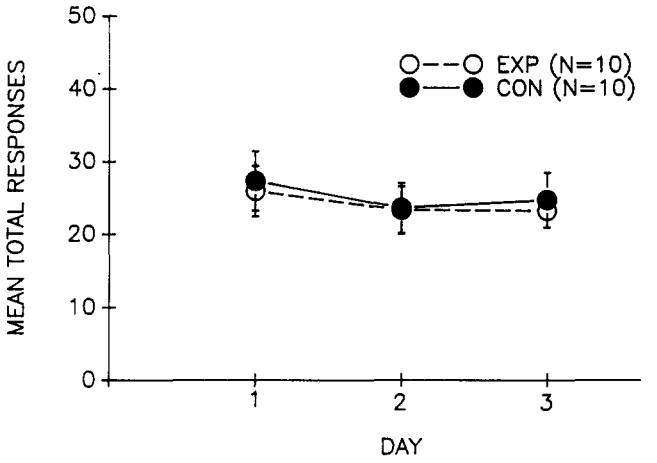

FIG. 3. Mean offensive plus defensive fighting responses ±SEM. The occasional absence of a SEM bar signifies that the SEM range was smaller than the circle indicating the mean.

produced by neonatal antidepressant drugs remains indetermined. Previous findings that REM sleep deprivation increased shock-induced aggression in rats (11) are consistent with the view that a permanent increase of REM sleep, or some correlate of it, diminished such aggression. It is of interest that like clomipramine, desipramine (1) and zimeldine (7) are strong REM sleep suppressants. Hence, if the lifelong increase of REM sleep produced by neonatal clomipramine is a permanent REM rebound in response to the drug's REM sleep deprivation at a critically early developmental stage, then neonatal desipramine and zimeldine may also produce a lifelong increase of REM sleep. In that case a permanent REM sleep increase may be a common effect of the three antidepressant drugs. On the other hand, the three tested antidepressant drugs affect different monoamines (3). Thus, parsimony would favor increased REM sleep or some correlate of it, rather than monoamine change, as a mediator of the behavioral alterations. Nevertheless, more direct experimental manipulations of hypothesized causal variables (REM sleep, monoamines) are necessary to test hypotheses about pathogenesis.

ACKNOWLEDGEMENT

This study was supported by NIMH grant No. MH40880-03.

REFERENCES

1. Baumann, P.; Gaillard, J.-M.; Perey, M.; Justafré, J.-C.; Le, P. Relationships between brain concentrations of desipramine and paradoxical sleep inhibition in the rat. J. Neural. Transm. 56:105–116; 1983.
2. Hicks, R. A.; Coleman, D. D.; Ferrante, F.; Sahatjian, M.; Hawkins, J. Pain thresholds in rats during recovery from REM sleep deprivation. Percept. Mot. Skills 48:687–690; 1979.
3. Hicks, R. A.; Paulus, M. J.; Johnson, J. C. Effect of REM sleep deprivation on electric shock threshold in rats. Psychol. Rep. 32:1242; 1973.
4. Hilakivi, L. A.; Hilakivi, I. Increased adult behavioral 'despair' in rats neonatally exposed to desipramine or zimeldine: An animal model of depression? Pharmacol. Biochem. Behav. 28:367–369; 1987.
5. Hilakivi, L.; Sinclair, J. D.; Hilakivi, I. Effect of neonatal treatment with chloripramine on adult alcohol related behavior in the rat. Dev. Brain Res. 15:129–132; 1984.
6. Hilakivi, L.; Stenberg, D.; Sinclair, J. D.; Kiianmaa, K. Neonatal desipramine or zimeldine treatment causes long lasting changes in brain monoaminergic systems and alcohol related behaviors in rats. Psychopharmacology (Berlin) 91:403–409; 1987.
7. Kupfer, D. Interaction of EEG sleep, antidepressants, and affective disease. J. Clin. Psychiatry 43:30–35; 1982.
8. Miczek, K. A.; Barry, H., III. Pharmacology of sex and aggression. In: Glick, S. D.; Goldfarb, J., eds. Behavioral pharmacology. St. Louis: The C. V. Mosby Co.; 1976:176–257.
9. Mirmiran, M.; van de Poll, N.; Corner, N.; Boer, G.; van Oyen, H. Lasting sequelae of chronic treatment with clomipramine during early postnatal development in the rat. IRCS J. Med. Sci. 8:200–202; 1980.
10. Mirmiran, M.; van de Poll, N. E.; Corner, H. G.; van Oyen, H.; Boer, G. Suppression of active sleep by chronic treatment with clomipramine during early postnatal development: effects upon adult sleep and behavior in the rat. Brain Res. 204:129–146; 1981.
11. Morden, B.; Conner, R.; Mitchel, G.; Dement, W.; Levine, S. Effects of rapid eye movement (REM) sleep deprivation on shock induced fighting. Physiol. Behav. 3:425–432; 1968.
12. Rosenthal, S. H.; Klerman, G. L. Content and consistency in the endogenous depressive pattern. Br. J. Psychiatry 112:471–484; 1966.
13. Vogel, G. W. A review of REM sleep deprivation. Arch. Gen. Psychiatry 32:749–761; 1975.
14. Vogel, G. W.; Vogel, F. A. A new animal model of human endogenous depression. Sleep Res. 11:222a; 1982.
15. Vogel, G. W.; Thurmond, A.; Gibbons, P.; Sloan, K.; Boyd, M.; Walker, M. REM sleep reduction effects on depression syndromes. Arch. Gen. Psychiatry 32:765–777; 1975.