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Repeated Pemoline Produces Self-Injurious Behavior in Adult and Weanling Rats

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MUELLER, K., E. HOLLINGSWORTH AND H. PETTIT. *Repeated pemoline produces self-injurious behavior in adult and weanling rats*. PHARMACOL BIOCHEM BEHAV **25**(5) 933–938, 1986.—Self-injurious behavior (SIB) is a serious problem among the mentally handicapped and is often accompanied by other repetitive or stereotyped behaviors. Acute administration of high doses of amphetamine or pemoline to rats produces transient SIB which is accompanied by severe deterioration of the behavioral repertoire. Repeated subcutaneous (SC) administration of pemoline to rats produces a high incidence of SIB without the dramatic behavioral changes produced by high doses of oral pemoline. Repeated pemoline increased locomotions and rears and produced intermittent stereotyped sniffing and licking/biting. However, the animals were still able to eat, drink, sleep and groom. Hotplate tests provided no evidence for analgesia. Because SIB is often associated with human developmental disorders, the effects of repeated SC administration of pemoline to weanling rats was also investigated. SC injections every 12 hours produced a high rate of SIB in weanling rats.

Pemoline Self-injurious behavior Stereotypy Hotplate test

SELF-INJURIOUS behavior (SIB) is a factor in approximately 20% of new admissions to residential institutions [7]. In addition to being fairly common among the retarded and autistic populations, SIB also tends to accompany the Lesch-Nyhan Syndrome [16], the deLange Syndrome [10,19], and occasionally, Tourette Syndrome [11]. Over the long term SIB is notoriously difficult to control. But in a few cases, neuroleptics are apparently useful [1,4]. SIB can also be produced in animals by administration of drugs. Properly used, such a preparation could be extremely useful in generating hypotheses for understanding SIB in humans.

Acute oral administration of high doses of pemoline to rats produces a high incidence of SIB. However, the SIB is accompanied by dramatic stereotypy. The animals remain in the same position for six hours or more exhibiting repetitive head movements and repetitive licking and biting of the cage. They are unresponsive to environmental changes and do not locomote in an open field until the stereotypy has subsided. The animals do not eat, drink, or groom for up to 18 hours after the administration of drug [14]. Furthermore, the SIB is temporary. SIB in humans tends to occur in episodes distributed over days or even months. And although human SIB also tends to be accompanied by repetitive behaviors, these stereotypies tend to be intermittent and may not be obvious for hours at a time.

The following studies attempted to improve the acute high dose pemoline model of SIB by producing intermittent but long lasting SIB without such an extreme disturbance of the behavioral repertoire. Pemoline, a long lasting indirect dopaminergic agonist [5,13], was repeatedly administered subcutaneously (SC) in oil (to retard absorption). Thus, drug was present more or less continuously over several days. Since human SIB is often considered as primarily a childhood problem, studies were also conducted with weanling rats. If drug induced SIB is a useful model of human SIB, one would expect to obtain drug induced SIB in weanling animals. Although SIB can be produced in weanling rats which have been neonatally treated with 6-hydroxydopamine [2,3] drug induced SIB has not yet been reported in intact weanling animals.

EXPERIMENT 1

METHOD

Animals

Rats were bred from Wistar stock at the TCU Department of Psychology animal facilities. Only male adults were used in Experiment 1. During the experiment the animals were housed in standard individual wire mesh cages with food and water present ad lib. The vivarium was maintained on a 12 hour light/dark cycle with behavioral testing always occurring during the light cycle.

Procedure

Pemoline was suspended in peanut oil and injected SC once daily with a large gauge needle. Several drug regimens were used in an attempt to produce SIB with only intermittent stereotypy; the drug regimens are summarized in Table 1. Controls were injected with an equivalent volume of oil, usually from 0.7 to 1.5 ml.

The animals were weighed daily and doses of pemoline were calculated daily. At the same time the animals were

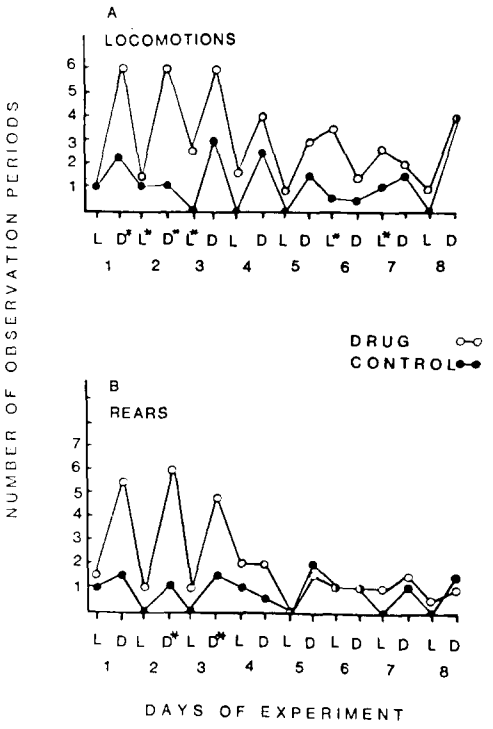

FIG. 1. The median number of 2-minute intervals in which animals exhibit locomotions (A) and rears (B). There are 10 2-minute intervals in both the light-cycle (L) and dark-cycle (D) observation periods. Open circles represent the data from the animals given repeated pemoline; closed circles represent the data from the animals given oil. *Denotes statistically significant difference between groups as determined by Kruskal-Wallis ANOVA.

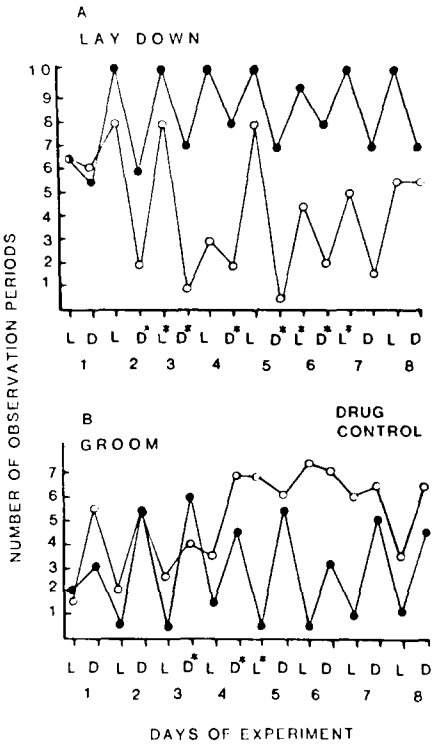

FIG. 2. The median number of 2-minute intervals in which animals exhibit "lay down" (A) and "groom" (B). There are 10 2-minute intervals in both the light-cycle (L) and dark-cycle (D) observation periods. Open circles represent the data from the animals given repeated pemoline; closed circles represent the data from the animals given oil. *Denotes statistically significant difference between groups as determined by Kruskal-Wallis ANOVA.

inspected for signs of SIB. Denuded skin, raw tissue, swelling, or bite marks were considered evidence of SIB. If an animal exhibited severe SIB (amputation of a digit or damage to muscle) that animal's dose of pemoline was reduced by half. This procedure was effective in preventing further tissue damage.

Animals were briefly observed 6 hours prior to injection and 3 hours after injection for obvious behavioral changes. In 2 studies (B and C) the animals were placed in an open field (66×57×30 cm divided into 9 areas) and "lines crossed" and "rears" were recorded for 3 minutes. During regimen B the open field test was conducted 96 hours after the last pemoline injection. During regimen C the open field test was conducted at both 24 hours and 96 hours after the last pemoline injection.

RESULTS

Animals in all regimens remained docile and easy to handle during the injections. Table 1 summarizes the body weight gained during the various drug regimens (as a general indication of the animals' health) and the incidence of SIB during the various regimens. Although regimen B (60-60-80-80-100-100-120-120-140 mg/kg/day) produced a high incidence of SIB accompanied by a reasonable body weight gain, two drugged animals died suddenly.

Regimen C (60-60-60-60-80-80-80-80-100 mg/kg/day) produced the highest incidence of SIB even though the total amount of pemoline injected was less than that injected in the other two regimens. Latencies to SIB ranged from 48 hours to 8 days after the first injection. The forefeet were the most frequent targets of SIB, but the hind feet, tail, shoulder, and penis were also targets. SIB usually lasted for several days and ranged from mild to severe.

Open field tests were conducted after regimens B and C. In both cases drugged animals crossed significantly more lines than controls (ANOVA, $p<0.05$). Animals given regimen C were also tested in an open field 96 hours after the last pemoline injection; there were no statistically significant differences between the two groups at that time (ANOVA, $p>0.05$).

EXPERIMENT 2

METHOD

Animals

Rats were raised and maintained in the same manner as in Experiment 1. Twelve male rats (mean body weight=503 g) were used.

Procedure

Six animals were injected SC with pemoline suspended in oil; six animals were injected with a similar volume of oil. Animals were injected daily at 3 hours before lights out ac-

TABLE 1

SUMMARY OF DRUG SCHEDULES FOR ADULT RATS

Drug Schedule (mg/kg/day)	Body Weight Gained During Experiment	Incidence of SIB
A. 80-80-80-80-80-80-80-80	9.3 g	2/8*
B. 60-60-80-80-100-100-120-120-140	19.0 g	7/8
C. 60-60-60-60-80-80-80-80-100	2.8 g	8/8

*The number of animals exhibiting SIB is shown in the numerator; the number of drugged animals is shown in the denominator.

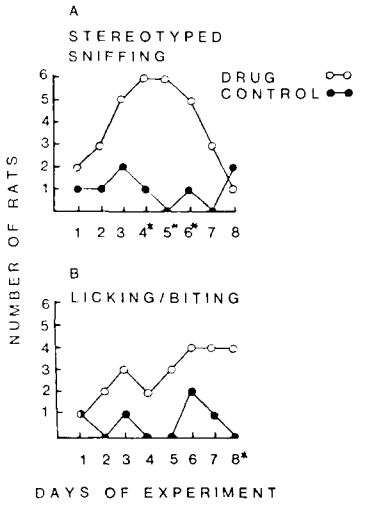

FIG. 3. The number of animals exhibiting stereotyped sniffing (A) and licking/biting of the cage (B) during at least two 2-minute intervals in a given day. Open circles represent the data from the animals given repeated pemoline; closed circles represent the data from the animals given oil. *Denotes statistically significant difference between groups as determined by the Fisher exact test.

cording to the following dose schedule: 60-60-60-60-80-80-80-80 mg/kg/day.

At 15 minutes after lights-out (3 hours after injection) and 5 hours after lights-on (20 hours after injection), home cage behavior was observed as described below. Rats exhibit well known circadian activity rhythms with activity peaks occurring during the midpoint of the dark and light cycles and during the transition between cycles. Thus both observation periods coincided with relative peaks in activity (although the activity peak occurring at the transition between cycles is greater than that during the midpoint of the light cycle).

A time-sampling procedure was used to quantify behavior. At each observation period each animal was observed during 10 2-minute intervals distributed over a 1.3 hour period. During each 2-minute interval the following behaviors were recorded as either present or absent: lay down (the rat does not support its body weight), groom (includes all preening and scratching), eat, drink, lick/bite (directed toward the cage), stereotyped sniffing (repetitive sniffing of the same location for over 10 seconds), locomotions, rears.

RESULTS

Four of the six pemoline-treated animals exhibited SIB with latencies ranging from 5 to 7 days after the first injection. The severity of tissue damage ranged from hair removal to bite marks and swelling. Targets of SIB included all four feet, the tail, and the abdomen.

As expected, drugged animals tended to locomote more than controls. The increased locomotions were most obvious during the dark-cycle observation period but occasionally hyperactivity did persist into the light-cycle (e.g., days 3, 6 and 7). The median number of observation periods in which locomotion was exhibited is shown in Fig. 1A.

In general the rearing data were similar to the locomotion data (see Fig. 1B). Although drugged animals reared more than controls in the early days of the experiment, by day 5 rears had returned to control levels.

As expected, "lay down" was recorded less often for drugged animals than for controls (Fig. 2A) and these differences often extended into the light-cycle. Nonetheless, drugged animals frequently exhibited "lay down," particularly during the light cycle.

Grooming occurred frequently in both controls and drugged animals (see Fig. 2B). After day 4 drugged animals tended to groom more than controls and circadian rhythmicity in this behavior disappeared.

Repetitive licking/biting of the cage was rarely observed. The median number of 2-minute observation periods in which licking/biting occurred was usually "0." Therefore, the number of animals exhibiting licking/biting during at least two 2-minute observation periods in a given day is shown in Fig. 3B. In general the same animal did not exhibit licking/biting throughout the duration of the experiment.

Stereotyped sniffing was not observed as often as expected. Therefore, the number of animals exhibiting stereotyped sniffing during at least two 2-minute observation periods in a given day is shown in Fig. 3A. Eating and drinking were rarely observed; these data are not shown.

EXPERIMENT 3

METHOD

Animals

Rats were raised and maintained in the same manner as in Experiment 1. Sixteen male rats (mean body weight=434 g) were used.

Procedure

Ten rats were injected SC with pemoline according to the dose schedule in Experiment 3. The remaining six rats were injected with a similar volume of oil.

A hotplate test was performed 24 hours prior to the first

TABLE 2

HOTPLATE LATENCIES OF CONTROLS AND ANIMALS EXHIBITING SIB

Day of Experiment	Group	Habituation Exposure	Test Exposure
Preinjection	OIL	23.71 ± 4.50*	19.46 ± 2.73
	SIB	24.19 ± 4.84	21.35 ± 2.25
4th day of injections	OIL	17.30 ± 2.57	16.03 ± 3.12
	SIB	18.09 ± 5.79	15.83 ± 1.93
8th day of injections	OIL	14.21 ± 3.73	9.92 ± 1.32
	SIB	13.28 ± 1.39	21.20 ± 5.31
3 days after last injection	OIL	13.56 ± 2.58	11.24 ± 1.84
	SIB	20.95 ± 5.04	11.42 ± 2.22

*Means and standard errors are shown. (ANOVA revealed no statistically significant difference between OIL and SIB on any day.)

TABLE 3

SUMMARY OF DRUG SCHEDULES FOR WEANLING RATS

Drug Schedule (mg/kg/injection)	Body Weight Gain During Experiment	Incidence of SIB
D. 60-60-70-80-90*	20.6 g	0/7†
E. 60-60-60-50-50-60-60-70-70	22.8	0/9
F. 50-50-50-60-60-60	13.6	6/11
G. 60-60-60-30-30	3.8	7/8

*In regimen D animals were injected daily. In all other schedules animals were injected every 12 hours. (Thus, both schedules D and E encompassed 5 days of injections.)

†The number of animals exhibiting SIB is shown in the numerator; the number of animals tested is shown in the denominator.

injection, 3 hours after the 4th and last injection and again 3 days after the last injection. The hotplate was maintained at 52 degrees centigrade. The latency to lick either the fore- or hindpaws was recorded. Data were always recorded from both a "habituation" exposure and a "test" exposure to the hotplate according to the procedure of O'Callaghan and Holtzman [17].

RESULTS

Eight of the 10 drugged animals exhibited SIB. Latencies to SIB were: 3rd day of injection (1 rat), 4th day of injection (4 rats), 5th day of injection (3 rats).

Hotplate latencies of the controls were compared to the latencies of animals exhibiting SIB (see Table 2) with analysis of variance. Differences between groups never attained statistical significance ($p>0.05$) during either the "habituation" or "test" exposure.

EXPERIMENT 4

METHOD

Animals

Rats were bred from Wistar stock at the TCU Department of Psychology animal facilities. Pups were weaned at 21 days postpartum. Both male and female pups from at least two different litters were used in each phase of the experiment. The weanling rats were allowed from 24 to 48 hours to habituate to standard individual cages; thus injections began at 22 or 23 days postpartum. In other respects the animals were maintained in the same manner as in Experiment 1.

Procedure

Pemoline was suspended in oil and injected SC in a volume of about 0.5 ml. Controls were injected with an equivalent volume of oil. Several drug regimens were used in an attempt to produce SIB accompanied by intermittent rather than intense and long lasting stereotypy; these regimens are summarized in Table 3.

RESULTS

No SIB occurred during regimen D (60-60-70-80-90 mg/kg/day). By 24 hours after the injection the behavior of drugged animals was indistinguishable from that of controls; therefore, during subsequent drug regimens animals were injected twice daily.

During regimen E (60-60-60-50-50-60-60-70-70 mg/kg/injection) the drugged animals became obviously hyperactive with respect to controls so the pemoline dose was temporarily reduced. SIB did not appear even when the dose was later increased.

During regimen F (50-50-50-60-60-60 mg/kg/injection) 6 of 11 drugged animals exhibited SIB with latencies ranging from 24 to 48 hours. Both forefeet and hindfeet were bitten. Although several animals exhibited severe SIB, virtually all SIB disappeared within 24 hours after its appearance and at that time healing had already begun. Males and females exhibited SIB with equal frequency.

During regimen G (60-60-60-60-30-30 mg/kg/injection) 7 of 9 animals exhibited SIB with all SIB appearing at 36 hours after the first injection. The dose of pemoline was reduced at that point in an attempt to prevent the SIB from becoming too severe. Targets of SIB were similar to those in regimen F and again both males and females were equally to exhibit SIB. These animals were unable to maintain body weight gain as well as animals in the other drug regimens (see Table 3).

GENERAL DISCUSSION

The main findings of this study are that pemoline can produce SIB without producing the dramatic disturbance of the behavioral repertoire that is associated with a high dose of acute oral pemoline [14] and without evidence of analgesia and that SIB can be produced in both intact adult and intact weanling rats with the same drug. These findings imply that SIB and other stereotypic behaviors may be mediated by different neurochemical mechanisms and that SIB need not represent a true developmental disorder.

SIB is reliably produced in rats by high doses of oral pemoline. However, the SIB always occurs during the in-

tense stereotypy phase of the pemoline response [14]. When placed in an open field these animals do not locomote but continue to exhibit SIB. SIB continues even when the animal is dipped in a beaker of water; undrugged animals struggle, shake, and groom. With acute pemoline SIB is almost always confined to the forelegs; such restricted targets of SIB may be another indication of intense stereotypy.

Such an extreme disturbance of the behavioral repertoire was never observed in the present study. Animals given repeated SC pemoline tended to locomote and groom more than controls. Intermittent stereotypy (stereotyped sniffing and licking/biting) did appear but in general the animals remained responsive to changes in the environment. SIB was intermittent, continued over a period of several days, and involved diverse areas of the body.

Of course the animals may have become progressively more stereotypic after the dark-cycle observation and may have gradually recovered by the light-cycle observation. However, several findings argue against this hypothesis. First, informal observations during pilot work found no evidence for intense stereotypy at any time after injection. Second, during the course of the dark-cycle observation the animals became progressively less active rather than progressively more stereotypic. Finally, the animals maintained steady body weights during the experiment. Had long periods of stereotypy occurred, one would have expected rather significant body weight losses to have occurred.

Thus the behavioral profiles of acute high dose pemoline and repeated pemoline have differences (e.g., intense stereotypy vs. intermittent low levels of stereotypy) as well as similarities (SIB). This is also true of the behavioral profiles of acute high dose amphetamine and continuous release amphetamine pellets. There have been occassional anecdotal reports of SIB occurring after acute administration of high doses of amphetamine and SIB is sometimes given the maximal value on scales rating amphetamine stereotypy [6, 8, 12, 18]. But like repeated pemoline, continuous release amphetamine pellets produce SIB with no evidence of intense stereotypy [9,15]. Thus one can conclude that SIB is not necessarily a high dose phenomenon and that SIB and intense stereotypy are mediated by different neurochemical mechanisms.

A common intuitive explanation for drug-induced SIB is that the drug produces analgesia and the analgesia somehow contributes to SIB. For example, both continuous amphetamine and repeated pemoline produce excessive grooming. In the presence of analgesia excessive grooming could lead to SIB. However, the hotplate test conducted in the present experiment provided no evidence for analgesia. Anecdotal reports suggest that human SIB is not necessarily accompanied by analgesia [16].

This is the first report of drug induced SIB in intact weanling rats. Intermittent SIB over a period of several days was much more difficult to produce in the weanling rats than in the adults. This problem may have stemmed largely from the anorectic effects of pemoline. An adult rat which gains weight at a slower rate than controls for about 10 days remains a healthy animal. But a weanling rat is in such a rapid growth phase that the slightest reduction in body weight gain can have serious consequences.

The studies with the weanling rats have implications for the etiology of SIB. One of the long-standing hypotheses about human SIB—particularly SIB which accompanies the Lesch-Nyhan syndrome, the deLange syndrome, etc.—is that some developmental event permanently alters the structure and/or function of the brain and allows SIB to occur. The ability of L-DOPA to produce SIB in neonatally 6-OHDA lesioned animals supports such a hypothesis [2,3].

The present research certainly does not contradict the developmental approach discussed above. However, it does point out that additional hypotheses should be considered. A transient functional change (e.g., induced by pemoline) appears to produce SIB as reliably as a permanent developmental (e.g., 6-OHDA lesion) functional or structural change.

In conclusion, repeated administration of pemoline to rats appears to offer a better model for human SIB than acute pemoline. This model may be useful for suggesting neurochemical mechanisms for human SIB.

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