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Chronic Administration of Typical, But Not Atypical Neuroleptics Induce Persisting Alterations in Rest-Activity Cycles in Rats

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ELLISON, G. AND R. E. SEE. *Chronic administration of typical, but not atypical neuroleptics induce persisting alterations in rest-activity cycles in rats.* PHARMACOL BIOCHEM BEHAV **36**(4) 807–811, 1990.—The behavior of rats administered chronic neuroleptics was observed using an extremely sensitive, computerized device which detected any cage movements, thereby continuously monitoring even very small levels of activity. In the first experiment, it was found that normal rats have a strong rest-activity rhythm with a cycle length of 60–70 min, whereas rats which have been chronically administered either haloperidol (HAL) or fluphenazine (FLU) decanoate for 20 months show a distinct lengthening of this cycle and that this effect persists long after cessation of drug injections. In a second experiment, it was further observed that these lengthened rest-activity cycles also occur when HAL is administered chronically either via osmotic minipumps or in the drinking water, but not following the chronic administration of two atypical neuroleptics (clozapine and raclopride). These findings suggest a useful new technique for the study of side-effects of neuroleptics in rats.

Chronic neuroleptics Animal models Haloperidol Fluphenazine Clozapine Raclopride Akathisia Chronopharmacology

THE chronic administration of neuroleptics to humans can induce several motor system side-effects, including the predominantly orofacial dyskinesias of tardive dyskinesia and the incessant restless movements of akathisia. Because long-term treatment with neuroleptic drugs in rodents has also been shown to increase the incidence of nondirected or "vacuous" chewing movements, this increased oral activity has been put forth as an animal model of tardive dyskinesia (5,16). However, there are several problems with this model of tardive dyskinesia. It can be argued (2, 9, 12) that the oral movements are not dyskinetic in character, that they appear too soon following the start of drug treatment, and that they tend to disappear rapidly upon cessation of drug treatment.

Utilizing a computerized detection device for automated measurement of mouth movements (4, 8, 13), we have found that rats given chronic neuroleptics over a period of six to eight months show gradual increases in the very smallest amplitude of computer-detected oral movements (OMs); these are of a size not generally detectable by human observers. However, it is not clear what these small OMs actually represent; it is possible that they reflect an inability on the part of the animals receiving chronic neuroleptics to keep any part of their body, including their mouth, still. In fact, while one of the most common and unpleasant side-effects of chronic neuroleptic administration in humans is akathisia, or an inability to remain still [and which may actually represent a tardive dyskinesia in the limbs; cf. (1)], there have been few attempts to measure akathisia-like phenomena in animals.

In an effort to determine whether rats administered chronic neuroleptics show any incessant low-level activity patterns, we developed a highly sensitive measure of general activity in rats using computerized detection of cage movements modeled after seismographic detectors. The present study tested the possibility that animals administered chronic neuroleptics might show an increased incidence of incessant, low-level activity. An unexpected finding was one of persisting alterations in rest-activity cycles (7) induced by typical, but not atypical neuroleptics.

METHOD

For testing, each animal was placed in one of four cages exactly like their home cage, but within a soundproofed room using the same reversed lighting cycle (12-hr on, 12-hr off) as their home cage. To allow for habituation, a period of 2 to 3 hours elapsed prior to data collection. These cages were suspended by four rigid springs from a very stable steel and cement frame resting on the concrete floor (each individual frame weighed approx. 80 kg). Each movement by the rat induced small displacements in cage position. These movements of the cage, which even at

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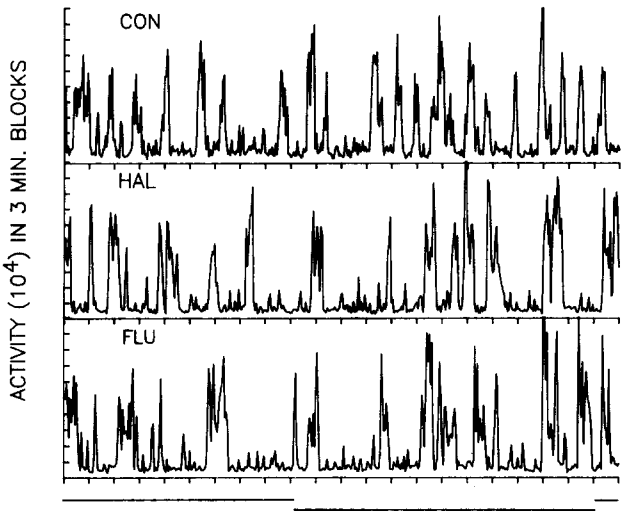

FIG. 1. Three typical recordings taken 2 months after last decanoate injection, following 18 months of chronic treatment. Each trace commences 2 hr after light offset in a control, a haloperidol decanoate, and a fluphenazine decanoate animal. Hour units of time represented by time marks, and the light cycle is indicated by event recorder mark, with "lights-on" being up. The activity units are arbitrary based on integrated output voltage, and are plotted as total voltage for each 3-min block.

maximal amplitude were barely discernable to the human eye, had a resonant frequency at 6–8 Hz.

Mounted to the steel frame and just adjacent (3 mm) to the side of each cage was a 4943 magnetic proximity detector probe (Electro Corp., Sarasota, FL) connected to a PBA200 amplifier. The outputs of these sensors, which detected any lateral movements of the cage, were amplified, fed to an analogue to digital converter board, and monitored via a computerized sampling circuit. At 60 times each sec, around the clock, the analogue output of each proximity detector was sampled by the computer and the absolute difference between the voltage of the present sample and the previous one was calculated and integrated over time. This provided a sensitive measure of any cage movement produced by the rat.

This measure of activity, representing the integral of total cage movement, was sorted each sec and placed into one of ten categories of activity. These were log categories ≤ 1, ≤ 2, ≤ 4, ≤ 8, ≤ 16, ≤ 32, ≤ 64, ≤ 128, ≤ 256, or >256 average activity units per computer cycle time, which was 60/sec. These categories were chosen based upon preliminary data indicating equally frequent categories over a 24-hr period. Every 3 min both the summated activity scores (total deflections in cage position throughout the 3 min), as well as the distribution of activity levels each sec (the 180 activity-sec scores in the 10 categories during that 3 min) were written to a file.

Several computer programs were developed which allowed for the determination of various parameters of the activity peaks recorded. A threshold was determined which specified a minimum amount of activity which defined an active animal. Based upon an examination of the data, this was set to 1,500 for the data reported here. A minimum width for a peak of activity was specified at 9 minutes for the data reported here and a minimum height for defining a peak was set at 2,000. The computer program then scored the average height of all peaks found, the average width of peaks, the number of peaks found, the average width of all interpeak periods of inactivity, and the average area for individual activity peaks. The data were examined by analysis of variance. Where significant main effects were found, post hoc comparisons between the control and experimental group were performed using Dunnett's tests.

Experiment I: Subjects and Drug Administration

Twenty-four female Sprague-Dawley rats (Simonsen, Gilroy, CA) initially weighing 234–297 g were divided into three groups of 8 each (two drug treatment groups and a control group) with initial body weights that were not significantly different. They were housed in individual cages under a 12-hr light/dark cycle with ad lib access to tap water and rat chow pellets (Wayne Lab Blox) except during behavioral testing. Treatment initially consisted of FLU decanoate, HAL decanoate, or vehicle (sesame seed oil), administered via IM injections given once every three weeks. Drug injections consisted of 21 mg/kg, giving an average daily dose of 1 mg/kg/day. The initial treatment period lasted eighteen months; then the animals were given a 2.5-month period when no drugs were administered, tested in the activity cages, injected with drugs or vehicle, and again tested between 7–10 days later.

Experiment II: Subjects and Drug Administration

In the second experiment, 36 rats were given various neuroleptics via their drinking water and then all animals were tested in the activity cages 6 and 20 weeks after initiation of drug treatment. The drug doses were selected to attain therapeutically equivalent amounts based on previous dosing strategies in rats (11,16). They were HAL (0.025 mg/ml), raclopride (RAC) (0.025 mg/ml), and clozapine (CLO) (0.5 mg/ml). Drug solutions were prepared fresh each week and intake recorded twice a week. Ethyl lactate was added to all solutions for flavoring (including control animals who received plain distilled water) at a concentration of 0.5 ml/liter. Water consumption (as a percentage of control intake) and actual drug dose range for each of the groups was HAL, 72% of control intake, 1.4–1.8 mg/kg/day; RAC, 101% of control intake, 1.6–2.3 mg/kg/day; and CLO, 65% of control intake, 24–32 mg/kg/day.

Experiment III: Subjects and Drug Administration

Experiment II was then repeated in a second set of animals using the same general procedures but with the drugs administered via subcutaneously implanted osmotic minipumps. In this experiment, the doses were HAL 1 mg/kg/day, CLO 15 mg/kg/day, RAC 1 mg/kg/day; all drugs delivered via an Alzet ML4 minipump left in the animals for 28 days. All animals were tested during the 4th week after pump implantation (i.e., between 21–27 days afterwards) and then again 7–10 days after pump removal.

RESULTS

Figure 1 shows typical recordings reflecting 20 hours of recording of activity from a control ("A"), a chronic HAL decanoate ("B"), and a chronic FLU decanoate ("C") animal. A highly distinct rest-activity rhythm can be seen, with peaks of activity occurring approximately every 60 minutes during the lights-off period and 69 minutes during the lights-on period in the controls (note that these are animals nearly two years old at the time of testing, for in younger animals the circadian rhythm is more pronounced and the rest-activity cycle less so). As can be seen in Fig. 1, there were significantly more activity peaks in the control animals compared to the neuroleptic-administered animals ($p<0.05$, Dunnett's test), even though these records were made 2 months following the last drug injection. Although the neuroleptic-treated animals showed less frequent cycles of activity, they

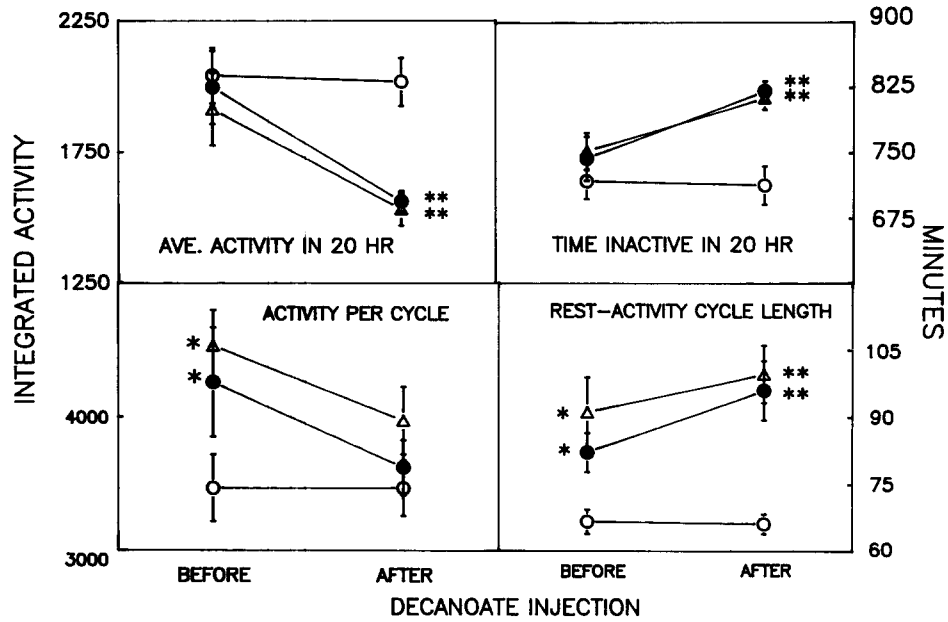

FIG. 2. Following extensive (18 months) repeated drug injections, the animals were given a 2.5-month drug-free period, tested in the activity cages ("BEFORE"), and then were given another injection and tested 7 to 10 days later ("AFTER"). This figure presents the results of computerized scoring of activity records throughout 20 hr of recording for controls (open circles), haloperidol (closed circles), and fluphenazine (open triangles) just "BEFORE" and one week "AFTER" drug or control injections. The left two graphs present integrated activity levels averaged across the entire 20 hr of recording (top) and averaged during each individual rest-activity cycle (bottom). The right two graphs depict average time inactive during the entire 20 hr (top) and average length of each individual cycle (bottom). *Means Dunnett's tests were significantly different from control, $p<0.05$; **means $p<0.01$.

had distinctly broader periods of activity (i.e., when they were active, they remained so longer than controls). Peak levels of activity did not distinguish between groups.

These longer rest-activity cycles in the drug-treated animals were seen both when the animals were tested 2½ months after depot injections and 10 days after depot injections. However, at 7–10 days after depot injections, the average activity was also significantly decreased in the neuroleptic animals, whereas it was not decreased 2½ months after injections. Figure 2 demonstrates these effects. The left two graphs show averaged integrated activity levels. The graph on the top left shows average activity throughout the 20 hr. While there were no significant differences between groups prior to the injection, $F(2,25)=0.26$, n.s., there were highly significant differences after the injection, $F(2,26)=15.2$, $p<0.001$, with both decanoate groups being significantly less active than the controls (both $p<0.01$, Dunnett's tests). This finding demonstrates the decreases in activity induced by the injections in the drug-treated groups. The top right graph of Fig. 2 shows total number of minutes "Inactive" during the 20 hr, again demonstrating decreased activity by the drugged animals which was also not significantly different prior to injection, $F(2,25)=0.65$, n.s., but highly significant after injections, $F(2,26)=13.3$, $p<0.001$, with both neuroleptic groups less than controls ($p<0.01$, Dunnett's).

The bottom left graph of Fig. 2 shows the amount of activity during each rest-activity cycle in the three groups. As can be seen, the drug-treated animals demonstrated more activity per cycle prior to injections, $F(2,25)=3.2$, $p<0.05$, an effect that decreased after injections. The bottom right graph illustrates why this is so. The average length of each cycle was dramatically longer in the drugged animals even 2½ months after their last injection, $F(2,25)=5.58$, $p<0.01$, and this effect is even greater 10 days after a further injection, $F(2,26)=11.7$, $p<0.001$.

These data were analyzed for the 10 categories of activity in order to determine if the chronic-neuroleptic animals showed an altered distribution of activity levels, and especially an inability to remain inactive during any part of the rest-activity cycle. From a 20-hour consecutive record, the 3-minute blocks were sorted so as to determine the 40 3-min blocks for each animal when it had been least active, and into the 40 blocks when it had been most active. Then, during these 40 blocks, the distribution of the 10 categories

TABLE 1

MEAN DURATION (MIN) OF REST-ACTIVITY CYCLES: EXPERIMENTS II AND III

	Control	Haloperidol	Clozapine	Raclopride	F(3,32)
After 6 weeks of oral drug	67.7 ±2.9	97.8 ±7.2	65.1 ±3.33	73.7 ±4.7	$F=9.6$ $p<0.0001$
After 20 weeks of oral drug	69.1 ±2.4	92.2 ±4.6	64.9 ±2.4	74.3 ±4.2	$F=11.5$ $p<0.0001$
On minipump	67.7 ±2.9	90.8 ±3.3	66.4 ±5.8	63.6 ±4.7	$F=6.5$ $p<0.002$
After pump withdrawal	70.5 ±6.1	79.1 ±4.0	63.3 ±2.8	64.0 ±3.4	$F=3.3$ $p<0.04$

of activity, from lowest to highest amount of activity during each sec, were calculated. It was thought that perhaps the control animals would show greater levels of activity during periods of greatest activity, but that an akathisia-like effect would be represented by an inability of the neuroleptic-treated animals to show extremely low levels of activity. No such interaction was found.

In a second experiment, groups of rats were administered neuroleptics in their drinking water and activity cycles studied 6 and 20 weeks after the beginning of drug administration. Table 1 shows that the main effect from the first experiment (i.e., increased duration of rest-activity cycles in the neuroleptic animals) was again replicated in the second experiment for the HAL animals. At both time periods the animals administered HAL had an increased rest-activity periodicity compared to controls, whereas rats administered the atypical neuroleptic CLO actually had a slightly decreased rest-activity cycle time and an increased number of peaks (not significant). Animals administered chronic RAC were not different from controls.

These effects were correlated with other alterations in activity. After 6 weeks of drug administration, only the HAL animals were significantly less active than Controls (Controls: 3692 ± 302 activity units; HAL: 2512 ± 87; CLO: 2940 ± 230; RAC 2846 ± 367); HAL group significantly less than both controls and atypical neuroleptics ($p<0.05$, Dunnett's test). Activity levels after 20 weeks of drug were similar but less significant, $F(3,32)=2.4$, $p<0.1$. The average width of active periods was also altered. After 6 weeks of drug administration, the average length of time active was 30.8 ± 1.6 min for Controls, 39.3 ± 3.7 min for HAL, 26.4 ± 1.5 min for CLO, and 30.7 ± 2.3 min for the RAC animals, $F(3,32)=4.8$, $p<0.01$, while by 20 weeks this same pattern was still significant [28.1 ± 1.8 min for Controls, 32.7 ± 2.6 min for HAL, 21.6 ± 1.0 min for CLO, and 24.6 ± 1.7 min for the RAC animals; $F(3,32)=6.7$, $p<0.001$]. At both time periods, the HAL animals were active for significantly longer than the Controls but the CLO animals were active for significantly shorter than the Controls ($p<0.05$, Dunnett's tests).

All of the effects described above were replicated in the experiment using continuous drug administration. In particular, there were significant differences in time between rest-activity peaks, $F(3,31)=6.5$, $p<0.002$, with the HAL animals showing significantly longer times than controls and the CLO animals again showing slightly shorter periods (see Table 1). This effect was still present 7–10 days after pellet removal, $F(3,31)=3.34$, $p<0.05$, even though the HAL animals were now significantly more active than the Controls ($p<0.05$).

DISCUSSION

This computerized method for detecting movements provided unique results concerning activity cycles in chronic neuroleptic-treated rats. The device used in the present study, which was modeled after sensitive seismographic recording equipment, monitored and precisely quantified movements of the animal by integrating over time all levels of activity. Even very minimal levels of inactivity could be scaled. In consequence, a pronounced rest-activity cycle, like that described for sleep cycles (3), was observed; this continued throughout the animals' day and night. The cycles we observed in rats using this sensitive activity measure (including a pronounced ultradian rhythm at about 60 min) are quite similar to those obtained monitoring respiratory activity, such as the continuous measurements of CO_2 expiration of rats (14). Our results are in further agreement with these authors that there is a much less pronounced circadian rhythm in aged animals than in younger animals. Thus, this sensitive but inexpensive technique for monitoring activity may prove to be a highly useful technique in studies of drug effects on activity rhythms and metabolic rate.

Using this procedure, it was found that there were distinct effects on activity produced by HAL and FLU, two drugs classified as "typical" neuroleptics based on their equipotent ability to block DA agonist-induced stereotypies and DA agonist-induced hyperlocomotion as well as their high incidence of motor side effects. (We have also obtained the lengthened rest-activity cycles in animals given chlorpromazine for 3 months in their drinking water.) While all neuroleptics show some degree of binding to the dopamine D-2 receptor site (6), certain neuroleptics have been labeled "atypical" due to a lower incidence of reported motor side effects and differential behavioral properties (15). Atypical neuroleptics, such as CLO and RAC, differ from traditional D2 blockers in that they only weakly inhibit DA agonist-induced stereotypies, while strongly blocking DA agonist-induced hyperlocomotion (10,15). They also lack the strong cataleptogenic effect seen with drugs such as HAL. As has often been reported, treatment with the typical neuroleptics HAL and FLU led to decreased overall activity levels, but by 2½ months after the previous decanoate injection, this overall effect on activity had largely disappeared. However, a second effect (which has not been reported previously) of these two neuroleptics was more persisting: a distinct lengthening of the rest-activity cycle. This finding was not due to the unique drug dosage or route of administration, for it was present following depot drug injections, and with osmotic minipump drug administration, and in animals given neuroleptics in the drinking water.

This effect of lengthened rest-activity cycles persisted much longer after a final depot injection than did the effects on overall activity. These results with HAL and FLU are congruent with the observation that across a variety of species the length of the rest-activity cycle is inversely correlated with basal metabolic rate. The lengthening effect on rest-activity cycles was clearly not present with CLO and RAC and, in fact, CLO actually induced the opposite effect (a shortening of this cycle).

Our results suggest that the effect on rest-activity cycles may be a more sensitive index of neuroleptic-induced sedation than is overall activity. Initially upon the administration of neuroleptics, it could not be measured, for the animals were highly inactive. However, after chronic neuroleptic administration, when the animals were tested after a prolonged period when decanoate injections were not given, while the overall effects on general activity had dissipated, the effect on the rest-activity cycle still persisted. Furthermore, when the animals were studied in drug withdrawal after chronic HAL, a time when the animals were actually overall hyperactive, the lengthened rest-activity cycles persisted. It is striking that, upon discontinuation of the typical neuroleptics, the altered rest-activity cycles persist while the hypoactivity reverses into the well-known phenomenon of discontinuation supersensitivity. This effect, then, appears to be a highly sensitive index of gradually developing and persisting effects of the chronic sedation induced by neuroleptics.

While the analysis of the distribution of activity scores sorted each second did not indicate that the rats administered chronic neuroleptics showed any tendency to be unable to remain inactive, it was clear that while the rats administered chronic HAL or FLU became active significantly less frequently than do controls, however, once active, they remained active for a longer period of time. This means that they actually show more activity during each cycle. It is possible that this altered rest-activity cyclicity is related to the akathisia reported in humans following chronic neuroleptics, however, we have not been able to find systematic recordings of akathisia in humans which could be compared with our rat data.

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