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Recording Oral Activity in Rats Reveals a Long-Lasting Subsensitivity to Haloperidol as a Function of Duration of Previous Haloperidol Treatment

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SEE, R. E., W. W. SANT AND G. D. ELLISON. *Recording oral activity in rats reveals a long-lasting subsensitivity to haloperidol as a function of duration of previous haloperidol treatment.* PHARMACOL BIOCHEM BEHAV **28**(2) 175–178, 1987.—Rats pretreated with no drug or with one of two dose levels of continuous haloperidol for 6, 12, or 24 weeks were then given a 5 month drug free interval followed by a single injection of 1 mg/kg haloperidol. Oral movement activity was recorded 2 days before and 7 days after the acute injection of haloperidol using a computerized scoring apparatus. Whereas prior to the acute injection there were no differences between groups, postinjection scores indicated a linear response curve, with the animals which had the least time of exposure showing the greatest increases in oral movement behavior. These data indicate that duration of treatment is a more important factor than is dose level in the development of persistent changes in dopamine-mediated oral activity.

Haloperidol Oral movement Tolerance

THE administration of neuroleptics can induce varied syndromes such as acute dystonias, or with more prolonged exposure, tardive dyskinesia. One of the primary symptoms of these disorders is the occurrence of abnormal mouth movements. Changes in dopamine receptor sensitivity were initially suggested as the basis for tardive dyskinesia [13], but subsequent analyses have emphasized long-lasting changes in GABA [6,7].

Chronic neuroleptic administration in rats has been shown to produce a syndrome characterized by an increase in vacuous chewing movements (VCMs) [14]. One of the questions raised concerning this proposed rodent model of tardive dyskinesia is whether these neuroleptic-induced changes persist, both behaviorally and biochemically, in rats (as they do in humans). One study which suggests a long-lasting effect in rats employed a novel paradigm. Gunne and Haggstrom [7] found an increase in VCMs following 8 months of haloperidol decanoate treatment. Five months after the last injection, a challenge dose of free haloperidol (i.e., the non-decanoate form) was administered. This injection increased VCMs to a level similar to that seen during the peak level at 8 months.

We have recently developed procedures by which VCMs in rats can be more precisely quantified using direct input into computer memory of televised measurements of mouth opening distance [5]. In the present study we focused on this reported phenomenon—of apparent long-term enhanced sensitivity to haloperidol—utilizing the paradigm employed by Gunne and Haggstrom.

METHOD

Seventy female, Sprague Dawley rats (Simonsen, Gilroy, CA), initially weighing 200–220 g were housed in single cages and given ad lib access to food and water. The rats were divided into seven groups of 10 each, consisting of 6 treatment groups and 1 control group. Rats in the drug-treated groups were administered haloperidol (HAL) base by means of subcutaneous, slow release silastic pillows similar to those described previously [4]. The pillows were constructed from 2.5×5 cm pieces of Dow Corning silicone sheeting, one 0.005 in. thick and the other 0.02 in. thick. The two pieces were fused along three outer edges and down the middle using a silicone adhesive and, after drying, filled with HAL base (obtained courtesy of McNeil Pharmaceuticals). In half of the pillows both chambers were filled, each with 50 mg ("High dose"), whereas in the other half only one chamber was filled with 50 mg HAL ("Low dose"). The pillows were then sealed along the final edge. After spreading the powdered base so that it was uniformly distributed within the silastic compartment, the pillows were implanted subcutaneously along the back of the animal just lateral to the vertebral column. Rats in the control group were implanted with empty pillows. All surgery was conducted under physical restraint after injections of lidocaine subcutaneously throughout the back region.

Haloperidol treatment lasted 6, 12, or 24 weeks at the two dose levels. Implantation was scheduled so that all pillows could be explanted at the same time. Release rates of these

TABLE 1

BASELINE ORAL ACTIVITY (COMPUTER-SCORED MOVELETS PER MINUTE) 2 DAYS PRIOR TO HALOPERIDOL INJECTION

	Treatment Duration (weeks)		
	6	12	24
HAL Dose			
High	113 ± 14.7	125 ± 11.4	109 ± 11.6
Low	101 ± 14.2	99 ± 16.1	104 ± 13.3
Control =	119 ± 13.3		

Mean ± S.E.M.; N=9–10 per group.

pillows were determined using HPLC analysis of the amount of drug remaining in the explanted pillows after various time periods compared to that in pillows filled with HAL at the same time, but not implanted in animals (stored in sealed containers). For this analysis, the pillows were opened and left in 50 ml of dilute lactic acid for two weeks; during this time they were periodically agitated until no traces of drug crystals could be observed. Then the HAL-lactic acid mixtures were diluted by taking 50 μl aliquots, diluting these in 100 cc of a phosphate-methanol buffer (recipe), and successive dilutions were made until the standard curve from the non-implanted pillows indicated a linear dose-response curve when injected in 50 μl samples onto a Hewlett-Packard HPLC using a 254 UV detector and an RP18 column.

Following explantation, a five month drug-free period was followed by a single injection of non-decanoate HAL given to all groups at a dose of 1 mg/kg. Oral movement activity in the rats was measured 2 days prior to and 7 days following the single challenge injection.

Oral movements were quantified using the following scoring procedure: all rats were habituated to being placed in plastic tubes (5.7 cm dia., 15.5 cm length) which rested inside a soundproof chamber. Following several sessions of habituation, struggling by the rat was minimal (less than 10% of total test time on average). At one end of the tube was a 3.3 cm hole through which the rat's head could protrude. On the upper and lower jaws of the rat small spots were painted using a UV sensitive dye. The chamber was illuminated only by a 6 W black-light bulb placed in front of and under the rat's muzzle. A closed circuit TV camera with a close-up lens and a UV filter was positioned 22 cm in front of the rat. The output from this camera was monitored by a human observer and also fed to a computer with a movement detection circuit (MM board, Biotronic designs, Tarzana, CA). This circuit calculated the distance (number of TV rasters) between the upper and the lower spot, and stored these data in computer memory 60 times each second throughout a 6 minute testing session. Oral activity was thus recorded as individual openings or closings of the jaw ("movelets"). Behaviorally, these movelets are characterized by vacuous chewing motions and/or rapid tremor of the jaws. The various movelets can be distinguished by their amplitude and slope and computer printouts of recorded oral activity can be examined to differentiate various types of oral movements. In the present study, movelets of all amplitudes were combined to give a recording of general oral activity. Movements of the head that altered the distance between the two dots were also recorded. Further details of this scoring system are reported in Ellison *et al.* [5].

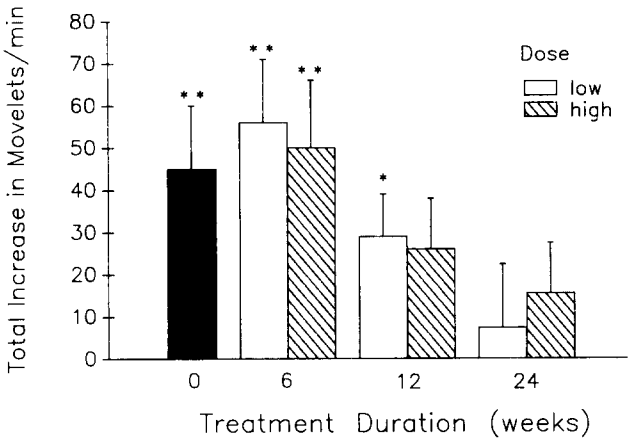

FIG. 1. Postinjection increase in oral activity. Average change in computer-scored openings and closings of the mouth (movelets/min) between the first and second behavioral recording session. The first recording session occurred 5 months after chronic drug treatment and 2 days before an acute injection of haloperidol (1 mg/kg). The second session was recorded 1 week after the acute injection. Data presented are means and standard errors of the mean; N=9–10 animals per group; *$p<0.05$; **$p<0.025$.

RESULTS

Analysis of the pillows removed from the rats indicated that the double chambered (100 mg) pillow released almost twice as much HAL as the single chambered (50 mg) pillow, and that release rate gradually decreased over time. Average HAL release rate in the 6 treatment groups was 24 weeks, High: 22.9 mg released; 24 weeks, Low: 11.25 mg released; 12 weeks, High: 12.3 mg released; 12 weeks, Low: 7.45 mg released; 6 weeks, High: 5.2 mg released; and 6 weeks, Low: 3.9 mg released.

Prior to initial pillow removal, while the animals were still receiving continuous HAL, vacuous chewing movements were monitored by a human observer using procedures described previously [12]. Increases in vacuous chewing movements over time were noted in all drug treatment groups, but did not persist following removal of haloperidol pillows (Sant, unpublished observations). During the period of chronic drug administration, HAL-treated animals showed an increase in body weight over controls, but these differences were not found at the time of the single challenge injection 5 months after cessation of drug treatment. During the 5 month drug free interval, 4 rats died of respiratory illness (1 control; 3 HAL-treated).

When tested initially in the computerized device, at 5 months after removal of the HAL pillows, there was no significant difference in oral activity across the seven groups [Table 1, $F(6,57)=0.357$, n.s.]. But on the second test, given 1 week later and following acute injection of HAL (1 mg/kg), there was a general increase in recorded activity in all groups.

Figure 1 shows this increase in oral activity (total number of computer-scored movelets over preinjection baseline level) at 7 days after the acute HAL challenge injection. Compared to results from the previous (preinjection baseline) test, total oral movement activity increased in all groups, but this was significant ($p<0.025$, t-test) only in the control group and the two 6 week groups, and in the 12 week, Low group ($p<0.05$, t-test). As can be seen in the figure, those drug groups which had received continuous HAL for

six weeks showed the largest increases in computer-scored movelets. Animals that received HAL for 24 weeks showed only minimal increases in activity, with the 12 week groups intermediate.

A linear trend analysis showed highly significant decreasing postinjection oral movement activity as a function of exposure time [planned comparison with isolated control; $F(1,57)=11.75, p<0.0025$] whereas the comparable comparison using drug dosage was not significant. It is interesting to note that several groups which received approximately the same amount of drug but over different time spans (6×High vs. 12×Low and 12×High vs. 24×Low) differed markedly in total movelet increase (Fig. 1).

None of the drug-treated groups in this study showed the increases in the smallest amplitudes of computer-detected oral movements which we have found in two studies [5,11] to be the most reliable index of gradually appearing effects of chronic neuroleptics.

DISCUSSION

These results indicate that animals with a previous history of having received prolonged treatment with HAL react differently from controls to a subsequent injection of HAL. However, contrary to the findings of Gunne and Haggstrom [7], the results of the present study indicate a trend in oral movement activity following acute injections of HAL in HAL-pretreated animals opposite to that predicted by a "tardive-dyskinesia reactivation effect." Thus, those animals with the least amount of previous exposure to HAL showed the greatest increases in oral movement activity, a pattern of results which does not suggest that this paradigm follows the pattern indicative of a model of reactivated tardive dyskinesia. Similarly, these animals did not show the profile of computer-detected movements which we have found indicative of tardive effects of neuroleptics (i.e., increases in the smallest amplitude of movements; see [5,11]).

However, these results are in the same direction as the findings reported in a recent experiment in which rat oral activity was observed in a similar test situation (Plexiglas tube) by a human observer [8]. Various antipsychotic drugs were given to drug-naive animals as single injections and oral movement activity was monitored just before and at several intervals after drug injection over a period of 7 days. Acute injections of two of the drugs used, HAL and fluphenazine, were found to produce a rebound increase over baseline measures in oral activity in drug naive rodents at 1–7 days postinjection. This pattern is similar to that seen in the control (drug-naive) and short-duration-treated animals in our present study, although our animals were not observed at numerous time points postinjection.

The present results do suggest a long-lasting effect of chronic neuroleptic administration. In the present study the post-HAL rebound effect was attenuated in rats which had previously received HAL, even though they had been drug free for 5 months, and furthermore the precise drug history of the animals apparently played a decisive role in determining the degree of attenuation of this effect. Thus, these results indicate that the time span of exposure to a neuroleptic plays a more important role than does the total dose administered in producing persisting pharmacological alterations as measured by changes in responsivity to an injection of HAL administered considerably later. These findings are in accord with results of other investigators who have examined long-term changes in dopamine-mediated behaviors in rodents treated chronically with neuroleptics. One common test for persistence of dopamine supersensitivity is the subsequent administration of DA agonists such as apomorphine. In a study by Clow *et al.* [3], rats treated with trifluoperazine or thioridazine for 6–12 months showed greatly enhanced stereotyped oral movement responses to apomorphine treatment, as well as increases in spontaneous oral activity, and these enhanced stereotyped responses persisted for three months after drug withdrawal. When apomorphine-induced stereotypy was used to examine the influence of duration of treatment in producing dopaminergic sensitivity [10], it was found that the amount of stereotypic responses increased directly with treatment duration in rats given chlorpromazine for 3, 6, or 9 weeks. The present results, when compared with these previous findings, indicate that following pretreatment with neuroleptics, administration of a DA antagonist produces a reaction which is opposite in some ways from that produced by a DA agonist challenge. Whereas a DA agonist enhances stereotypic activity to a greater degree in animals pretreated with a DA antagonist, pretreatment with a DA antagonist reduces the rebound effect of increased oral activity in animals following HAL, a finding which provides complementary evidence for persistent changes following drug treatment.

The direction of this effect is of a very long-lasting tolerance, the degree of which is dependent on the total time of previous neuroleptic exposure, a result which is especially interesting because there are few previous reports of such long-lasting neuroleptic effects [1,2], and because some behavioral measures indicate that tolerance to neuroleptics does not develop. Yet, with the procedures used here, a long-term tolerance phenomenon appears to develop after chronic HAL treatment.

Although others have suggested the importance of DA antagonist dosage in producing DA supersensitivity [9], the present study found no significant differences between two different dose levels. Although the use of a wider dose range might better elucidate the role of dosage in producing changes in stereotyped responses, these results from animals imply that short-term, high dosage drug therapy might have less persistent side-effects than prolonged administration of lower doses.

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