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Contingent Suppression of Tolerance to Haloperidol: A Dose-Response Analysis

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WOLGIN, D. L. *Contingent suppression of tolerance to haloperidol: A dose-response analysis.* PHARMACOL BIOCHEM BEHAV 35(2) 273–280, 1990.—Rats were given injections of haloperidol (HAL; 0.625 or 2.5 mg/kg) either before (Before groups) or after (After groups) access to sweetened milk on alternate days. Controls (Saline groups) were given injections of saline. At biweekly intervals ("test days"), all groups were given pretest injections of the drug in order to monitor the development of tolerance in the After and Saline groups. Rats in the Before groups showed no tolerance to the initial suppression of milk intake. In contrast, rats in the After groups had greater intakes, although the level of intake declined on subsequent test days in the group given the lower dose. Rats in the Saline groups drank less on the test days than any of the other groups, suggesting that sensitization occurred. These results are consistent with previous findings (29) that tolerance to HAL is suppressed following pretest injections of the drug. The degree of suppression appears to be inversely related to the frequency of such injections.

Haloperidol Behavioral tolerance Sensitization Dosing schedule Anhedonia
Reinforcement density hypothesis Motor impairments Drug cumulation Rats

A growing body of evidence suggests that, under appropriate conditions, behavioral variables contribute to the development of tolerance to drugs [see, e.g., (12) for a recent review]. One such variable is reinforcement (24). For example, several studies have shown that subjects become tolerant to the behavioral effects of amphetamine and other stimulants when the initial effect of the drug results in a loss of reinforcement, but not when the drug increases, or has no effect on, the frequency of reinforcement [see, e.g., (6, 8, 10, 24, 31)]. However, this is not invariably true, as recent studies with alcohol have shown (18,20). Thus, the conditions under which the development of tolerance follows the "reinforcement density hypothesis" (9) remain to be elucidated.

A link between tolerance and reinforcement loss suggests that tolerance may be mediated by instrumental learning. That is to say, if the initial effect of a drug results in a loss of reinforcement, the subject may learn an appropriate coping response in order to regain the lost reinforcements. An example of this phenomenon is the ability of amphetamine-treated rats to suppress stereotyped head-scanning movements that interfere with their ability to drink milk from a drinking tube (30). In general, however, there is little direct evidence for the role of instrumental learning in tolerance development [see (28) for a recent review]. Instead, learning is inferred from the association between tolerance and reinforcement loss.

One implication of the proposal that tolerance involves instrumental learning is that if a drug blocks the rewarding effects of an otherwise reinforcing stimulus, learned tolerance should not develop. This prediction was recently confirmed in a study involving the effects of haloperidol (HAL), a drug that is thought to block dopaminergic circuits that control reinforcement (26,27). In this study, rats given daily injections of HAL prior to having access to milk showed little tolerance to the initial suppression of feeding even after 54 days of treatment. Surprisingly, rats given daily injections of the drug *after* they had already ingested the milk developed substantial tolerance, which was revealed when they were subsequently tested with pretest injections (29). Thus, the suppression of tolerance was contingent on having access to milk while intoxicated.

The finding that tolerance was suppressed in rats given milk while intoxicated suggests that drug-induced "anhedonia" interfered with the development of learned tolerance. The development of tolerance in the group given posttest injections is more difficult to interpret. One possibility is that tolerance develops when drug administration is not associated with reinforcement loss. For example, tolerance develops to the initial cataleptic and biochemical effects of neuroleptics (2, 4, 5, 11, 17, 22). However, because the doses in this study were relatively high (2.5 and 5 mg/kg) and the drug was administered daily, it is possible that rats given posttest injections were slightly intoxicated as a result of drug cumulation when they were given access to milk. If this were the case, then a learning interpretation of tolerance would still be tenable, at least at the presumably lower drug concentrations found in these rats.

In the following experiments, an attempt was made to control for this possibility by giving the drug on alternate days and monitoring milk intake on the intervening days, when the rats were given injections of saline. If drug cumulation occurs, then baseline intake on these intervening days should decline. In addition, in

order to assess the development of tolerance more accurately, two additional features were incorporated into the design. First, dose-response curves were determined before and after chronic exposure to the drug. Second, the rate of tolerance development in rats given posttest injections was assessed at biweekly intervals, rather than at the end of the chronic regimen. As will be shown below, the results of these experiments provide additional evidence that tolerance to HAL is suppressed when drug treatment occurs in the context of feeding.

EXPERIMENT 1: EFFECTS OF 2.5 mg/kg

In the first experiment, an attempt was made to replicate the results of the previous study (29) using the 2.5 mg/kg dose of HAL, but with the procedural refinements described above.

METHOD

Subjects

The subjects were 21 male Sprague-Dawley albino rats weighing 380–482 g at the start of drug treatment. The rats were housed individually in stainless steel cages under a 12-hr alternating light/dark cycle (lights on 0600 hr) and maintained on three Purina Lab Chow pellets (about 15 g) and ad lib water daily, except where otherwise indicated.

Procedure

Establishing baseline intakes. In order to establish baseline levels of milk intake, the rats were given daily 30-min tests in their home cages during which water bottles were replaced with calibrated drinking tubes containing sweetened condensed milk diluted with water (1:2). These tests were conducted over 27 days; during the last 9 days, the subjects were injected with isotonic saline (1 ml/kg, IP) 30 min before access to the milk. At the end of each test, water bottles were replaced and the rats were fed.

Chronic phase. The subjects were then assigned to one of three groups (n's = 7) matched for body weight and milk intake. During the chronic phase of the experiment, rats in each group were given two injections each day, one 30 min before, and the other 30 min after, the 30-min test. On drug days, the Before group received pretest injections of HAL (2.5 mg/kg) and posttest injections of saline. The After group received these injections in the reverse order, i.e., pretest injections of saline and posttest injections of HAL (2.5 mg/kg), whereas the Saline control group received injections of saline both pre- and posttest. Drug trials were conducted on alternate days. In order to control for potential differences in milk intake on drug days produced by the different treatment conditions, the intakes of rats in the After and Saline groups were yoked to those of the rats in the Before group. This was accomplished by staggering the trials by one drug day so that the former groups were offered the mean amount of milk ingested by the Before group on the previous drug day.

On the intervening nondrug days, all groups were given injections of saline both before and after the session. On these days, unlimited milk was given to all of the groups in order to monitor their baseline levels of intake. During the course of the experiment, one rat from the Before group developed an overgrown incisor, which interfered with its ability to eat pellets and lick the drinking tube. This rat's data were excluded from the statistical analysis.

Test days. In order to monitor the development of tolerance in the After group, rats in this group were tested with pretest injections of HAL (and posttest injections of saline) at approximately 2.5-week intervals during the chronic phase of the experiment. These tests were conducted on regularly scheduled drug days in lieu of the usual posttest drug treatment. Because intermittent pretest drug experience may, in itself, either promote or inhibit tolerance, rats in the Saline group were also tested with pretest injections of HAL and posttest injections of saline on the same days. If biweekly pretest injections of drug promote or inhibit tolerance, then the Saline group would be expected to show such effects as well.

Dose-response determinations. Dose-response curves were determined both before and after the chronic phase of the experiment. For the initial dose-response determination, the rats were given pretest injections of saline and each of 5 doses of HAL (0.156, 0.312, 0.625, 1.25, and 2.5 mg/kg) in a random order. Drug tests were separated by 3–6 days during which the rats received injections of saline both before and after access to milk. For the second dose-response determination, the rats were given pretest injections of saline and each of 6 doses of HAL (all of the previous doses plus 5 mg/kg). In order to maintain the level of tolerance acquired during the previous phase of the experiment, each group continued to receive its respective drug treatment on alternate days, but test doses were substituted for the usual treatment every other drug day. Thus, test doses were separated from each other by three trials (an intervening saline day, the chronic drug treatment, and another intervening saline day).

Drugs

HAL was obtained from McNeil Pharmaceuticals, Spring House, PA in the form of Haldol Injection (5 mg/cc) and diluted, when necessary, with physiological saline. All doses were injected in a volume of 1 cc/kg except for the 2.5 mg/kg dose, which was injected in a volume of 0.5 cc/kg.

Data Analysis

The data were analyzed by means of Student's *t*-tests and by analyses of variance (ANOVA) for repeated measures. When warranted by significant F ratios, individual comparisons were made with Tukey's Honestly Significant Difference test.

RESULTS

Chronic Phase

Mean milk intake during the chronic phase of the experiment is presented as 3-day blocks in Fig. 1. As indicated in the upper panel, intakes during the first block of saline trials ranged from 22–24 ml. Over the course of the experiment, however, the intakes of the groups diverged, as confirmed by a significant group × block interaction, $F(38,342) = 3.58$, $p<0.001$. By the last block, the intake of the After group had decreased by about 4 ml, the intake of the Saline group increased by about 5 ml, whereas the intake of the Before group remained about the same, despite a decrease in intake over the first 6 blocks. Although the differences between the first and last blocks for the After and Saline groups were small, they were statistically significant, $t(6) = 2.77$, $p<0.04$ and $t(6) = 3.15$, $p<0.02$, respectively.

The effect of chronic pretest injections of HAL is shown in the bottom panel of Fig. 1. On the first block of drug trials, rats in the Before group ingested only 9% of the amount they consumed on the previous block of saline trials. No tolerance developed to this effect during the course of the experiment [e.g., the difference between the first and last blocks was not significant, $t(6) = 1.26$, $p>0.05$].

Test Days

The mean amount of milk consumed on the 7 test days, when

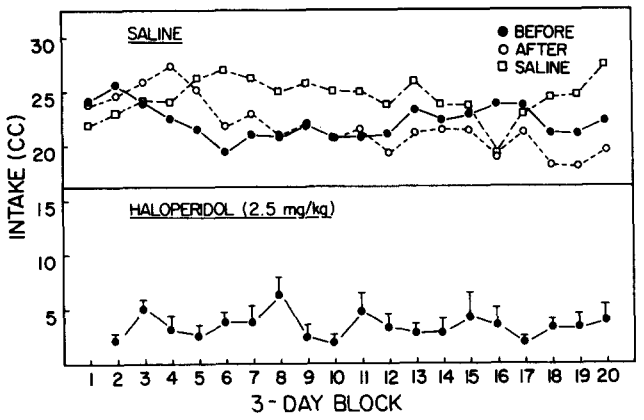

FIG. 1. Mean milk intake of rats in the Before, After, and Saline groups following injections of saline (top) and of rats in the Before group following injections of 2.5 mg/kg HAL (bottom). Each data point represents the mean of 3 trials.

all groups were given pretest injections of HAL (2.5 mg/kg), is presented in Fig. 2. For purposes of comparison, the mean intake at this dose during the initial dose-response determination is also indicated. Analysis of the data revealed a significant main effect of group, $F(2,18)=5.47$, $p<0.02$, but a nonsignificant effect of day and a nonsignificant group × day interaction. Collapsing across days, the After group ingested significantly more milk than the Before and Saline groups, and the Before group ingested more milk than the Saline group (cf. Fig. 2, inset). It is interesting to note that the After group drank more than the other groups even though its intake on the intervening saline days declined slightly during the course of the experiment (cf. Fig. 1). Finally, the After group drank more on the first test day than it did when previously given this dose during the initial dose-response determination [9.71 vs. 1.57 ml; $t(6)=3.82$, $p<0.009$].

As shown in Fig. 2, the Saline group ingested less milk on the test days than either of the other groups. By test day 6, rats in the Saline group had also gained more weight during the course of the experiment than those in the other groups, despite the yoking procedure (mean weight gain: 14, 9, and 34 g for the Before, After, and Saline groups, respectively). Because milk intake on the test days might be influenced by body weight level, rats in the Saline group were given only 1 Purina pellet daily (instead of the usual 3 pellets) during the 2-week interval between test days 6 and 7 in order to bring their body weights into line with those of the other groups. During this period, rats in the Saline group lost an average of 52 g, whereas rats in the Before and After groups gained 4 and 7 g, respectively. Despite this change in deprivation level, milk intake on the last test day was not different from that on the previous test days. Similarly, milk intake on the saline trials between test days 6 and 7 was not significantly different from that on the block of trials preceding test day 6.

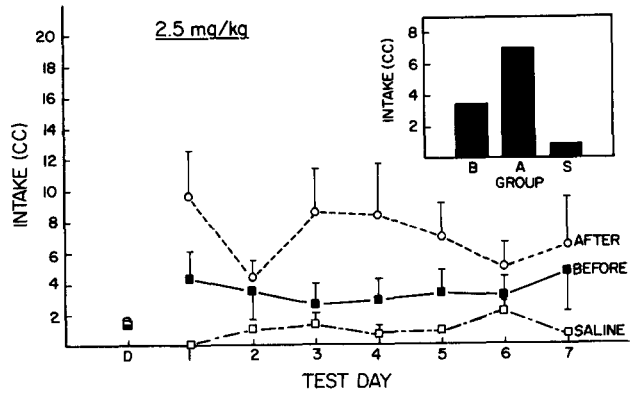

FIG. 2. Mean milk intake (±S.E.) of rats in the Before, After, and Saline groups following pretest injections of 2.5 mg/kg HAL on each of the 7 test days. For purposes of comparison, intakes at this dose during the initial dose-response determination are also indicated (D). Inset: Mean milk intake averaged across all 7 test days.

Because the test days represented the first exposure of the Saline group to chronic HAL (other than during the initial dose-response determination), its milk intake on those days was compared to that of the Before group on the first 7 drug trials of the chronic phase. In such a comparison, the groups are equated in the number of pretest injections they received, but differ in the frequency of the injections. Statistical analysis revealed a significant effect of group, $F(1,12)=29.27$, $p<0.001$, but a nonsignificant group × day interaction. Post hoc tests showed that the Saline group drank less milk during the test days than the Before group did during the first 7 trials of the chronic phase (marginal means: 0.96 and 3.53 ml, respectively).

Dose-Response Determinations

The mean amount of milk consumed by each of the groups during the dose-response determinations is shown in Fig. 3. In order to evaluate potential differences between dose-response assessments, the data for each group were analyzed by separate ANOVA's, with dose-response determination (initial vs. final) and dose (0, 0.156, 0.312, 0.625, 1.25, and 2.5 mg/kg) as factors. There were no differences between the dose-response determinations for the Before and Saline groups. However, this was not the case for the After group, as evidenced by a significant dose-response determination × dose interaction, $F(5,30)=3.00$, $p<0.025$. Inspection of Fig. 3 suggests that intakes differed under saline, and this was confirmed by statistical analysis, $t(6)=3.72$, $p<0.01$. In order to control for the effect of this baseline shift, the intakes for each dose-response determination were converted to percentages of saline intakes (see Table 1) and the data were then reanalyzed. Statistical analysis of the converted scores revealed that the two dose-response determinations did not differ, i.e., neither the main effect of dose-response determination nor the interaction was significant.

Incidental Observations

Three types of motor impairment were observed on days that the rats received injections of HAL. First, unless they were drinking, the rats were relatively immobile throughout the session. Typically, they remained in a prone position, often without postural support, and with their eyes closed. In general, the Saline group was more impaired than the other groups on the test days. Second, HAL-injected rats licked the drinking tube at a slower rate than undrugged rats [cf. (13)]. This effect was most evident on days when the Before group was given HAL and the After and Saline groups were given saline, when direct comparisons could be made. However, even on test days, when all of the rats were given HAL, this deficit was clearly evident. Moreover, HAL-injected rats often missed the drinking tube with their tongues as they attempted to drink. This deficit appeared to be the result of an impairment in tongue protrusion. Finally, all of the rats developed repetitive oro-bucco-lingual movements [cf. (23)]. These move-

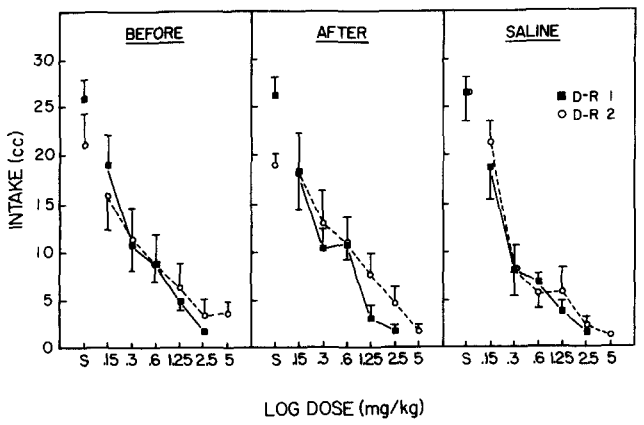

FIG. 3. Mean milk intake (±S.E.) of rats in the Before, After, and Saline groups following injection of saline (S) and each of various doses of HAL during the initial (D-R 1) and final (D-R 2) dose-response determinations.

ments appeared immediately following injection and terminated several minutes later, before the rats had access to milk. They were first observed in the Before and After groups during the first week of chronic drug treatment, but eventually they were observed in all three of the groups. Interestingly, these movements also occurred immediately following injection on the intervening saline days, suggesting either that they had become conditioned or that they were elicited by the arousal associated with the injection.

DISCUSSION

In this experiment, tolerance was assessed at two points in time: During the chronic phase, when the rats were tested with a standard dose of the drug, and later, when the dose-response curves were redetermined. Rats in the Before group were not tolerant to the initial suppression of intake induced by HAL at either point in time. In contrast, rats in the After group did become tolerant initially, as indicated by their milk intakes on the test days. However, such tolerance was not accompanied by a shift to the right of the final dose-response curve. The reason for this inconsistency is not readily apparent, but it is possible that with the additional pretest injections, tolerance was suppressed by the anhedonic effect of the drug.

In comparing the results of this experiment with those reported previously (29), it should be noted that the level of tolerance in the previous experiment was considerably greater. One reason for this difference may lie in the schedule of drug injections. In the previous experiment, the drug was given daily. Because the intakes of the After groups were yoked to those of the Before groups, this schedule resulted in gradually increasing levels of food deprivation during the course of the experiment, which may have counteracted to some extent the anhedonic effects of the drug. In contrast, in the present study, the drug was given on alternate days with intervening trials in which the rats had unlimited access to milk for 30 min. Consequently, the rats may have been less hungry on drug days in the present experiment. This interpretation is supported by an analysis of the body weight changes in the two experiments. In the previous study, the rats in the Before and After groups lost weight, whereas in the present case they gained weight.

One unexpected finding of this experiment was that the Saline group showed a greater suppression of intake on the test days than the Before group did on its first seven exposures to the drug. The significance of this finding will be discussed later (see the General Discussion section).

The intermittent schedule of drug injections employed in the present experiment permitted the continuous monitoring of the baseline level of milk intake. This procedure revealed that rats in the After group showed a small but reliable baseline shift toward the end of the experiment. This shift in baseline might reflect the gradual accumulation of HAL in body tissues. It should be noted, however, that rats in the Before group, which had a similar history of drug exposure, did not show a parallel shift in baseline intake. Thus, unless the accumulation of HAL in body tissue is differentially affected by when the injection is given relative to feeding, drug cumulation does not appear to offer a satisfactory explanation of the data. Nevertheless, in the following experiment a lower dose of HAL was administered chronically in an attempt to further minimize the potential contribution of drug cumulation.

TABLE 1

MILK INTAKE OF AFTER GROUP

	Dose (mg/kg)				
D-R	0.156	0.312	0.625	1.25	2.50
1	70 ±14	39 ±7	41 ±5	11 ±4	6 ±3
2	105 ±29	73 ±22	58 ±12	43 ±14	28 ±12

Values represent mean intakes (±S.E.) during initial (1) and final (2) dose-response determinations (D-R) expressed as a percentage of saline intake.

EXPERIMENT 2: EFFECTS OF 0.625 mg/kg

This experiment was a replication of the previous one except that rats in the Before and After groups were given a dose of 0.625 mg/kg HAL chronically in order to minimize the likelihood of drug cumulation.

METHOD

Subjects

The subjects were 21 male Sprague-Dawley albino rats housed and maintained exactly as in Experiment 1.

Procedure

The procedures were identical to those described in the previous experiment except that 0.625 mg/kg haloperidol was given to the Before and After groups on alternate days and to all three of the groups on the test days.

RESULTS

Chronic Phase

Mean milk intake during the chronic phase of the experiment is presented as 3-day blocks in Fig. 4. As shown in the upper panel, intakes ranged from 23–25 ml on the initial block of saline days. However, by the last block of trials, milk intakes declined slightly in the After and Saline groups (by 5.2 and 3.7 ml, respectively), but not in the Before group. Although small, the reductions in intake for the former groups were statistically significant, $t(6) = 4.88$, $p<0.003$ and $t(6) = 2.92$, $p<0.03$, respectively. All of the

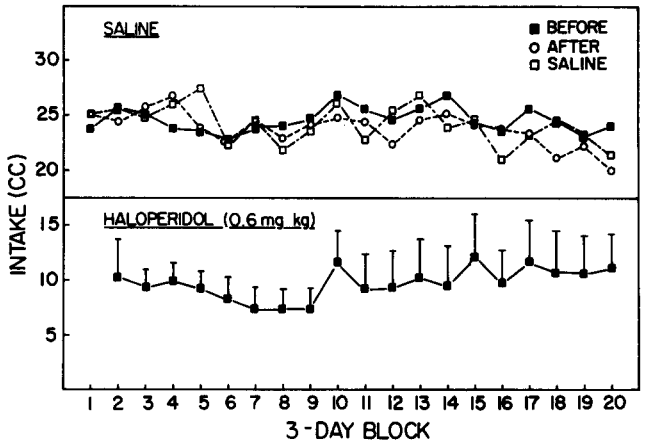

FIG. 4. Mean milk intake of rats in the Before, After, and Saline groups following injections of saline (top) and of rats in the Before group following injections of 0.625 mg/kg HAL (bottom). Each data point represents the mean of 3 trials.

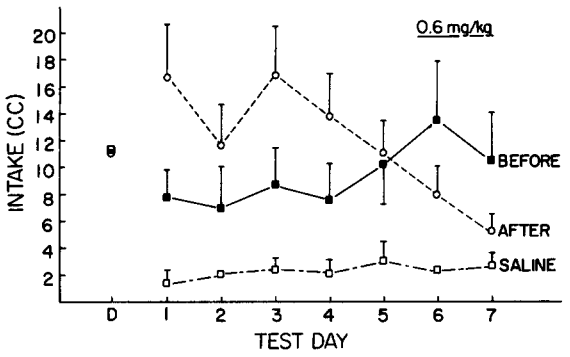

FIG. 5. Mean milk intake (±S.E.) of rats in the Before, After, and Saline groups following pretest injections of 0.625 mg/kg HAL on each of the 7 test days. For purposes of comparison, intakes at this dose during the initial dose-response determination are also indicated (D).

groups gained weight during the course of the experiment, and there were no differences between the groups (mean weight gains: 84, 85, and 72 g for the Before, After and Saline groups, respectively).

The bottom panel of Fig. 4 shows the effect of chronic pretest injections of HAL. On the first block of drug trials, rats in the Before group drank only 43% of their intake on the previous block of saline trials. Again, no tolerance developed to this effect over the course of chronic treatment, $F(18,90)=1.04$, $p>0.05$.

Test Days

The mean amount of milk consumed on the 7 test days, when all groups were given pretest injections of HAL (0.625 mg/kg), is shown in Fig. 5. For purposes of comparison, the mean intake at this dose during the initial dose-response determination is also indicated. Statistical analysis confirmed a significant group × day interaction, $F(12,102)=3.18$, $p<0.001$. On the first test day, the After group drank more milk than either the Before or Saline groups, although their intake did not differ from that on the initial dose-response determination. By the last test day, however, milk intake dropped significantly [Day 1 vs. Day 7, $t(6)=3.35$, $p<0.02$]. On test days 4–7, the intake of the After group was not significantly different from that of the Before group, and on test days 6–7 it was not significantly different from that of the Saline group. Although the milk intakes of the Saline group did not differ from that of the Before group during the first 5 test days, their intakes were significantly lower on the last 2 days.

It is clear from Fig. 5 that the Saline group's milk intake was consistently low on each of the test days. Because the Saline group was tested with HAL only on these days, it was of interest to compare the intake of this group with that of the Before group on its first 7 trials with HAL during the chronic phase. As previously described, in such a comparison, the groups are equated in the number of pretest injections they received, but differ in the frequency with which the injections were given. Statistical analysis of the data revealed a significant difference between the groups, $F(1,11)=13.59$, $p<0.003$, but a nonsignificant group × trial interaction. Collapsing across trials, the Saline group drank less milk than the Before group (mean intake: 2.3 and 10.3 ml, respectively).

In order to further examine this effect, the milk intakes of the Before, After, and Saline groups on their first two exposures to HAL (0.625 mg/kg) were compared. In all three groups, the first test occurred during the initial dose-response determination. For the Before group, the second test occurred on the first day of the chronic phase, whereas for the After and Saline groups it occurred several weeks later, on the first test day. As shown in Table 2, the milk intake of the Saline group was significantly lower on the second exposure to this dose of HAL than on the first, whereas the intakes of the Before and After groups on their first two exposures were not statistically different.

Dose-Response Determinations

The mean amount of milk consumed by each of the groups during the dose-response determinations is shown in Fig. 6. For the Before group, there was a significant dose-response determination × dose interaction, $F(5,25)=3.50$, $p<0.02$, suggesting that the two curves were not parallel. Although inspection of Fig. 6 suggests that differences might be found under saline and at the 1.25 mg/kg dose, Tukey post hoc tests failed to reveal significant differences at these, or at any other, doses. This anomaly appears to be the result of a relatively large error term associated with the main effect of dose-response determination, which is part of the pooled error term used in the post hoc tests. However, a t-test also failed to reveal differences at the 0 and 1.25 mg/kg doses.

A significant dose-response determination × dose interaction

TABLE 2

MILK INTAKE FOLLOWING INJECTION OF 0.625 mg/kg HALOPERIDOL

Exposure		Group Before	After	Saline
1	Mean	11.17	11.14	11.43
	S.E.	±2.37	±2.49	±2.03
2	Mean	6.83	16.71	1.43*
	S.E.	±3.00	±4.03	±0.97

Values represent mean intakes during the initial dose-response determination (Exposure 1) and on the following exposure to that dose (Exposure 2).

*Less than on Exposure 1, $t(6)=3.72$, $p<0.01$.

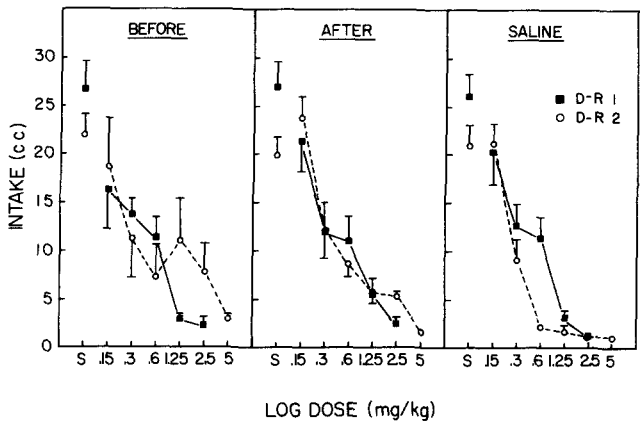

FIG. 6. Mean milk intake (±S.E.) of rats in the Before, After, and Saline groups following injection of saline (S) and each of various doses of HAL during the initial (D-R 1) and final (D-R 2) dose-response determinations.

was also found for the After group, $F(5,30)=3.99$, $p<0.007$. Again, Tukey post hoc tests failed to reveal significant differences between any of the doses. In this case, however, a t-test indicated that rats in the After group drank significantly less milk under saline on the final dose-response determination than they did on the initial one, $t(6)=4.54$, $p<0.004$. This finding is consistent with the results from the chronic phase of the experiment, which also indicted a baseline shift in this group. Because such a shift complicates the interpretation of differences between the dose-response determinations, a second analysis was performed after first standardizing the data by converting the intakes at each dose of HAL to a percentage of intake under saline (see Table 3). Again, a significant interaction was obtained, $F(4,24)=3.36$, $p<0.025$. Post hoc tests revealed that milk intake under the 0.156 mg/kg dose was greater on the final dose response determination than on the initial one. No other differences were significant.

For the Saline group, there was a significant main effect of dose-response determination, $F(1,6)=12.48$, $p<0.02$, but a non-significant dose-response determination × dose interaction. Inspection of Fig. 4 suggests that the difference in dose-response determination was the result of decreased milk intakes under saline and at the 0.625 mg/kg dose on the final determination. Although the interaction term was not significant, t-tests confirmed a significant difference at the 0.625 mg/kg dose. This result is consistent with the data presented in Table 2, which shows that intake on the first test day was also significantly less than that on the initial dose-response determination.

TABLE 3

MILK INTAKE OF AFTER GROUP

	Dose (mg/kg)				
D-R	0.156	0.312	0.625	1.25	2.50
1	78 ±5	43 ±7	41 ±6	22 ±4	8 ±3
2	129* ±21	67 ±19	47 ±10	32 ±10	34 ±15

Values represent mean intakes (±S.E.) during initial (1) and final (2) dose-response determinations (D-R) expressed as a percentage of intake under saline.

*Exceeds intake on D-R 1, $p<0.05$.

Incidental Observations

Although the symptoms were not as severe, all of the groups showed the three types of motor impairment described in Experiment 1. On drug days, the rats were relatively immobile, and on the test days, rats in the Saline group were more impaired than those in the other groups. In addition, the rate of licking appeared to be slower than normal, and most of the rats developed dyskinetic oral movements during the course of the experiment.

DISCUSSION

In many respects, the results of this experiment were similar to those of Experiment 1. Rats in the Before group did not become tolerant to the initial suppression of milk intake produced by HAL. In contrast, rats in the After group ingested more milk on the first test day than any of the other groups. However, milk intake in the After group gradually declined during the remaining test days. This effect was similar to the gradual extinction of food-rewarded operant responding in neuroleptic-treated rats reported by Wise *et al.* (27), and suggests that intermittent exposure to the pretest injections of HAL resulted in the suppresion of intake.

However, despite the use of a lower dose of HAL, evidence of a baseline shift was found once again. If this effect was the result of drug cumulation, then the lack of tolerance in the Before group, as well as the loss of tolerance in the After group, cannot be attributed unambiguously to the anhedonic effect of the drug. Although the possibility of drug cumulation cannot be dismissed, several findings suggest that it did not play a major role. First, although the Before and After groups had a comparable history of drug injections, the Before group did not show a significant baseline shift during the intervening saline days. Moreover, the loss of tolerance by the After group during the test days was not matched by a parallel decline in the Before group. If drug cumulation occurred, it is difficult to see why it would affect the After group, but not the Before group. Finally, the Saline group also showed a shift in baseline during the intervening saline trials, although it received injections of HAL only at biweekly intervals. It is unlikely that the drug accumulated over this long an interval. Instead, it is possible that the shifts in baseline observed in this experiment were caused by an attenuation of the reinforcing effect of the milk as a result of the previous history of drug-induced anhedonia.

As in Experiment 1, the Saline group exhibited a greater suppression of milk intake on the test days than either of the other groups. This finding must be considered in assessing the degree of tolerance in the Before and After groups. At the 0.625 mg/kg dose, rats in these groups did not drink more on the first test day than they did on the initial dose-response determination. Thus, by one criterion, at least, they were not tolerant. However, rats in the corresponding Saline group, which had a similar history of pretest drug exposure, showed sensitization to this dose of the drug on the first test day. Consequently, the absence of sensitization in the Before and After groups must be attributed to the series of injections that they received between the initial dose-response determination and the first test day. In this sense, their intakes on the latter reflects the development of tolerance. Even so, the intake of the Before group was suppressed relative to that of the After group.

GENERAL DISCUSSION

In a previous study (29), we reported that rats given daily injections of HAL prior to a milk-drinking session showed little tolerance to the initial suppression of intake, whereas rats given

the drug after milk access for the same period of time showed substantially more tolerance when they were subsequently tested with pretest injections of the drug. It was proposed that rats given pretest injections did not learn to overcome the initial suppression of feeding because drug-induced anhedonia blunted the reinforcing effect of the milk. In contrast, rats given posttest injections did not experience such anhedonia because they did not have access to milk while they were intoxicated. They did, however, acquire a presumably unlearned form of tolerance as a result of chronic exposure to the drug.

One problem with this interpretation is that, because rather large doses of the drug were given daily, drug cumulation may have occurred. Consequently, rats given posttest injections may have been intoxicated somewhat when they were given milk, and, therefore, may have become tolerant as a result of learning. From this perspective, the differential development of tolerance in the Before and After groups might be attributed to differences in drug concentration at the time of testing. That is to say, learned tolerance may have occurred in the After groups because drug levels at the time of testing were relatively low, whereas such tolerance did not develop in the Before groups because drug levels were too high. The present studies were, therefore, designed to minimize drug cumulation and to provide a means of determining whether any "carry-over" effects occurred.

Consistent with the previous findings, rats given pretest injections of the drug showed no recovery of intake during the course of chronic testing. In contrast, rats given posttest injections, particularly at the 2.5 mg/kg dose, showed substantial milk intake as early as the first test day. Although some changes in baseline intake were noted, particularly in the groups given the higher dose, these effects tended to occur toward the end of the experiment. Thus, tolerance observed on the first reversal day cannot reasonably be attributed to the effect of drug cumulation. As explained above, several other findings also argue against an interpretation of the results in terms of drug cumulation, although the possibility cannot be ruled out entirely.

Haloperidol also produced several motor deficits, which appeared to affect the rats' ability to drink. However, there were no apparent differences between the Before and After groups in this regard on the test days. For example, both groups were immobile except when they were drinking. Moreover, in the previous study (29), in which the motoric effects of the drug were systematically monitored, no differences were found between the Before and After groups. Consequently, differences in milk intake between these groups cannot be explained in terms of the direct motor effects of the drug. An alternative explanation for the failure of the Before groups to become tolerant is that sensorimotor feedback associated with movement (licking) was aversive. It has been proposed that the conditioning of such aversiveness to feeding-related stimuli contributes to the suppression of responding for food (25). In the present context, however, it is unlikely that conditioning would have occurred because both drug and saline injections were given in the same environment. Moreover, such an explanation cannot account for the even greater suppression of intake in the Saline groups, which received drug injections only biweekly.

In one important respect, the results of this experiment differed from those of the previous one. In the previous study, there was no loss of tolerance in the After groups when they were switched to pretest injections of the drug at the conclusion of the experiment. This was not true in the present case. Rats tested with the lower dose (0.625 mg/kg) showed a marked decrease in intake durng the course of the test days. Moreover, neither of the After groups were tolerant at the conclusion of the chronic phase, when the dose-response curves were redetermined. This loss of tolerance may have resulted from the series of pretest injections given on the test days. Such an effect, of course, is consistent with the view that tolerance to HAL is suppressed by the anhedonic effect of the drug when injections are given prior to feeding. However, it is unclear why a similar effect was not found in the previous experiment [cf. (29)]. Moreover, this interpretation does not explain why rats in the Before groups did not also show a gradual decline in intake during the test days. Finally, it is important to note that other studies involving the effects of neuroleptics on ingestive behavior have also reported findings that are inconsistent with an anhedonia interpretation [e.g., (3, 14, 15, 19)].

One variable that may help to explain some of these disparate findings is the schedule of injection. As previously noted, the Saline groups, which were injected with HAL only at biweekly intervals, ingested less milk during the test days than did the other groups. Moreover, the Saline groups drank less on these trials than the Before groups did on their first seven exposures to the drug during the chronic phase of the experiment. In fact, the Saline group tested with 0.625 mg/kg drank less on the first test day than it did on the only previous time it was tested with that dose, during the initial dose-response determination. A similar effect could not be confirmed for the Saline group tested with 2.5 mg/kg because intake on the initial dose-response determination was already quite low.

The difference in milk intake between the Before and Saline groups cannot be attributed to the development of tolerance in the Before groups, because their intakes were not significantly different from those on the initial dose-response determination. Rather, it appears that the Saline groups became more sensitive to the drug. Although baseline intake also declined somewhat in these groups, the increased sensitivity occurred on the first test day, at which time baseline intakes were normal. For the Saline group tested with the lower dose, the increased sensitivity was also reflected in a downward shift in the final dose-response curve. However, because this effect was restricted to the 0.625 mg/kg dose, it does not appear to represent sensitization in the pharmacological sense, which is characterized by a shift to the left of the entire dose-response function. It is unlikely that drug cumulation can account for these findings because the Saline group received injections of HAL at 2.5 week intervals.

These results suggest that there is an inverse relation between the frequency of pretest injections of HAL and the degree of suppression of milk intake. The Saline groups, which were injected biweekly, showed the greatest degree of suppression. Indeed, as previously explained, these groups exhibited sensitization to the "anorexic" effect of the drug. The Before groups, which were injected on alternate days, had stable, low intakes, which tended to be higher than those of the Saline groups. These groups showed neither tolerance nor sensitization. The After groups, which received both pre- and posttest injections, showed a more complex pattern, however. These groups initially had the highest intakes, suggesting that posttest injections promote tolerance to the drug. In rats given the lower dose, milk intakes subsequently declined, presumably as a result of the biweekly pretest injections. Although there was no significant decline in rats given the higher dose (2.5 mg/kg), tolerance was lost by the second dose-response determination.

It is interesting that higher doses of HAL (i.e., 2.5 and 5 mg/kg) were also used in the previous experiment (29), in which no loss of tolerance was observed in the After groups when they were switched to pretest injections. One reason may be that injections were given daily, not intermittently. However, it is also possible that sensitization of HAL-induced "anorexia" is inversely related, not only to the frequency of pretest injections, but also to the dose of the drug. Because these two variables determine the concentration of the drug in the brain, it may be inferred that sensitization is more likely with low tissue levels of HAL. This is

consistent with the more general proposal that sensitization is often associated with intermittent drug administration whereas tolerance is associated with continuous administration (21).

Several lines of evidence support the view that the behavioral effects of chronic neuroleptic administration are dependent on the schedule of injections. For example, in the anhedonia literature intermittent injections of neuroleptics result in a gradual decrease in food-rewarded responding [e.g., (27)], whereas daily injections can result in tolerance to the initial suppression of responding (15, 16, 29). Similarly, intermittent injections of HAL produce sensitization to the initial drug-induced reduction of locomotor activity, whereas twice daily injections produce a trend toward tolerance (7). Finally, although tolerance occurs to the cataleptic effect of HAL when the drug is given daily (11), sensitization occurs after only two intermittent exposures to the drug, and this "time-dependent sensitization" is greater the longer the interval between the injections (1).

In conclusion, the results of the present series of experiments confirm that tolerance to HAL's effect on feeding is suppressed in rats given pretest injections of the drug under conditions in which drug cumulation is minimized. In addition, they suggest that the degree of suppression is inversely related to the frequency of such injections. To the extent that the suppression of feeding induced by HAL represents an underlying anhedonic effect of the drug, these results support the view that reinforcement plays an important role in the development and maintenance of tolerance.

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