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Clozapine does not induce a motor impairment in operant responding for heat reinforcement

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Abstract

This experiment examined the effects of intraperitoneal (i.p.) clozapine (CLZ) compared to haloperidol (HAL) on operant responding for heat reinforcement in a cold environment ($-8°C$). Three doses of CLZ (1, 3 and 5 mg/kg) were found to dose-dependently increase responding for heat while lowering core temperature (T_c) only at the highest dose. Three doses of HAL (0.1, 0.3 and 0.5 mg/kg) dose-dependently decreased operant responding which resulted in a dose-dependent decrease in T_c. The highest dose of CLZ was then tested in two other paradigms: a reinforcement schedule in which heat was available as long as the lever was held down, and a temperature gradient (range 7–45°C) in which access to heat required minimal motor effort. The ad libitum reinforcement schedule still did not provide enough heat to overcome the hypothermic effects of CLZ. However, in the gradient, rats receiving CLZ selected a warmer region of the gradient, and T_c was higher than that of controls. These data support CLZ's reputation for having minimal motor side effects. Unlike HAL, the hypothermic effects of CLZ appear to be unrelated to effects of the drug on movement.

Keywords: Clozapine; Thermoregulation; Haloperidol; Motor deficits; Rat

One of the most vexing problems in the search for a satisfactory treatment for schizophrenia has been the extrapyramidal side effects (EPS) associated with the use of classic neuroleptic drugs. These compounds are effective in alleviating positive psychotic symptoms associated with the disease (delusions and hallucinations), but they also produce a host of extrapyramidal symptoms (Parkinsonian-like movements and tardive dyskinesia) [9]. The source of both the efficacy of these drugs and EPS is relatively clear. It is well established that the compounds impart a majority of their anti-psychotic effects via antagonism of dopamine (DA) at D_2 receptors (although other transmitter systems are believed to be involved as well). However, because sufficient levels of striatal DA are necessary for proper motor functioning, these drugs also induce EPS [11]. The incidence and severity of EPS is not trivial, occurring in as many as 75% of schizophrenics receiving standard neuroleptic treatment, and EPS is a frequent cause of neuroleptic-induced noncompliance with prescribed dosage regimens [2]. Not surprisingly, much pharmaceutical research on schizophrenia during the last 40 years has focused on developing drugs that will ameliorate the symptoms of this disease without producing deficits in motor functioning.

The classic neuroleptic drugs disrupt motivated behaviors in both laboratory animals and humans. The hypothesis is that by blocking mesolimbic DA receptors, these drugs act to reduce the rewarding impact of external stimuli (i.e., make them less reinforcing) [4]. In humans receiving standard neuroleptic treatment, this manifests itself as anhedonia, or blunted affect. In animal models, this effect is exhibited as drug-induced reductions in operant responding for rewarding stimuli such as food, water, sex and brain stimulation [14]. However, because standard neuroleptics are also known to produce marked bradykinesia, the possibility always exists that these reductions in operant responding are simply the result of a lessened capacity to perform the operant task [4]. Researchers have attempted to separate these reward effects from performance effects by employing multiple operant tasks that vary in regard to the degree of motor output they require. The results of such experiments indicate that standard neuroleptics may promote reductions in operant

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responding via reward effects or performance effects, or both, depending on the nature of the reinforcer [4].

There is now a new generation of antipsychotic drugs called "atypical" antipsychotics. These drugs are notable because they treat the symptoms of schizophrenia without causing EPS and tardive dyskinesia. One primary example of an atypical antipsychotic is clozapine (CLZ). CLZ has been found to not cause significant EPS [10] as opposed to typical neuroleptics such as haloperidol (HAL). However, CLZ does resemble standard neuroleptics in that it acts as a potent hypothermic agent in both animal models [8,13] and humans [7] and this inherent hypothermic property offers a unique opportunity to examine the effects of this drug on heat reinforcement.

Rats placed into a cold ambient temperature (T_a) and given access to a lever that activates heat lamps will operantly respond to maintain their core temperature (T_c) [1]. Past work has demonstrated that pretreatment with standard neuroleptics disrupts leverpress responding for heat in a cold T_a, resulting in a reduction in T_c. This same study demonstrated that this effect is due to a drug-induced deficit in motor capacity as opposed to a motivational deficit [3].

In the present study, we replicate this finding with the classic neuroleptic HAL, and compare the effects to those of CLZ. Given that CLZ induces hypothermia, but is not believed to disrupt motor functioning, the hypothesis tested is that CLZ will increase the behavioral demand for heat in a cold T_a, but that the motor problems associated with typical neuroleptics will not be present. Three different tests were used that each demanded a different amount of motor effort. A brief 3-s heat reinforcement, which was contingent upon a leverpress, required continuous effort to obtain heat. The second test provided heat as long as the lever was held down, thus requiring less motor effort. Finally, a thermal gradient test was used in which heat could be obtained with almost no motor effort.

1. Method

1.1. Animals

Two groups of 10 female Sprague–Dawley rats were obtained from Charles River Laboratories. The animals were housed individually in hanging wire cages, and fed Purina Chow (5001) and water ad libitum. The colony room was maintained at 22°C with a relative humidity of 50% and a light:dark cycle of 12:12 (lights on 0700 h); all tests were conducted during the light phase of the cycle.

1.2. Drugs

CLZ HCl was a gift from Sandoz Laboratories to Dr. Seiji Nishino (Department of Neuroscience and Psychiatry, Stanford University). It was dissolved in 4% acetic acid (concentration 20 mg/ml) and diluted with saline. HAL was purchased from Research Biochemicals (Natick, MA). It was dissolved in 4% acetic acid also and diluted with saline. Acetic acid (4%) diluted with saline (one part acetic acid to two parts saline) served as the control vehicle. All injections were intraperitoneal (i.p.).

1.3. Leverpress apparatus

The test apparatus allowed animals to obtain heat in a cold environment by pressing a lever in order to activate infrared heat lamps. A circular 22-cm diameter and 22-cm deep wire mesh cage was equipped with a 3×4 cm^2 Plexiglas lever which protruded 5 cm into the cage 2 cm above the floor. Two 250-W red bulb infrared lamps were mounted at each side of the cage at a 45° angle to the floor and focused on the rat at the lever. The power dissipated by the lamps was set at 300 W, which produced an irradiance of 180 mW/cm^2, as measured by an Eppley thermopile. The apparatus was placed in a 0.48 m^3 freezer maintained at -8 ± 2°C. A 25-W red incandescent lamp provided illumination. Pressing the lever activated the heat lamps. Equipment in an adjoining room provided a cumulative record of the pattern of responding as well as the number of leverpresses and the cumulative duration of heat lamp activation.

1.4. Leverpress procedure

The animals were shaved closely with an Oster clipper the day prior to a test. The reason for shaving the animals was to reduce insulation and thus prevent sporadic performance that occurs due to piloerection. The rats were trained to press the lever in order to activate the heat lamps, and then given at least four additional trials of 90 min duration so that both operant respondings for heat and body temperature were stable for two consecutive tests. The standard test was to allow 30 min of baseline responding in order to permit adaptation to the test conditions, and to obtain a measure of colonic temperature (T_c) maintained by the behavior in the absence of drug treatment. The animal was removed from the test apparatus after the 30-min baseline, and T_c was measured with a Physitemp (Clifton, NJ) BAT-12 meter and thermocouple probe inserted 7 cm. The drug was then injected, and the animal returned to the apparatus for an additional 60 min. T_c was again measured on removal from the test. The animals were tested twice per week with 3–4 days intervening between tests.

1.5. Gradient apparatus

The apparatus consisted of a Plexiglas cylinder (180 cm in length and 7 cm in diameter). The entire length of the cylinder was wrapped with copper tubing, through which hot (50°C) water was pumped by means of a Lauda B-1 pump. The apparatus was housed in a cold room maintained at 5°C. The resulting temperature with-

in the gradient ranged from 45°C at the warm end to 7°C at the cold end. A scale marked on the gradient was visible from outside the cold room. The temperature corresponding to this scale was calibrated by measuring the temperature along the gradient's inner length with a Yellow Springs Instruments meter (no. 46) and thermistor probe (no. 402) encased in 25 g of clay. The position of the animal was recorded during a test and then converted to a temperature based on the calibration.

1.6. Gradient procedure

The animals were given two to three adaptation tests in the gradient before drug testing began. Temperature preference was determined by observing the position of the animal and the amount of time spent at that position for 10-min intervals. This position preference was converted to a temperature preference weighted for time at any location. The standard test procedure following adaptation was to place the animal in the gradient for 40 min prior to injection of drug or vehicle. The position of the rat was recorded during the last 20 min of this period as the baseline temperature preference. The animal was then removed and T_c measured. The rat was injected with the drug for that test and returned to the gradient for 60 min of observation. The animal was then removed and its T_c measured again.

1.7. Protocols

Experiment 1 examined the effects of CLZ and vehicle in the leverpress apparatus at a T_a of -8°C. Ten trained rats were given vehicle and CLZ in a counterbalanced order. Each rat eventually received saline, vehicle and 1, 3 and 5 mg/kg of CLZ on different days to establish a dose–response curve for the drug. Both saline and vehicle were tested to determine if the vehicle alone had any effect. In this experiment, the leverpress apparatus was set for a constant reinforcement duration of 3 s of heat for each leverpress. Responses made during a reinforcement had no effect. The total number of responses and reinforcements was counted; the difference between the number of responses and reinforcements is responses emitted during a reinforcement. Body weight (±SEM) averaged 272.8 (±5.8)g during these trials.

Experiment 2 examined the effects of HAL and vehicle in the leverpress apparatus. The procedure was identical to that of Experiment 1, except that each rat eventually received vehicle and 0.1, 0.3 and 0.5 mg/kg of HAL. A fixed duration reinforcement (FDR) of 3 s was used for this experiment as well so that a comparison between HAL and CLZ could be made. Mean body weight was 281.5±5.75g. One group of rats was used for Experiments 1 and 2 and a second group of 10 rats was used for Experiments 3 and 4.

Experiment 3 examined the effects of CLZ and vehicle in the leverpress apparatus again. However, this time, the reinforcement schedule was changed so that the heat lamp would stay on as long as the lever was held down. Ten rats were given a fixed dose of 5 mg/kg of CLZ based on the dose–response curve results. Mean body weight was 259±2.8 g.

Experiment 4 examined the effects of CLZ and saline in the gradient apparatus. Ten rats were given CLZ and saline at a fixed dose, again, of 5 mg/kg in a counterbalanced order. Mean body weight was 305±10 g.

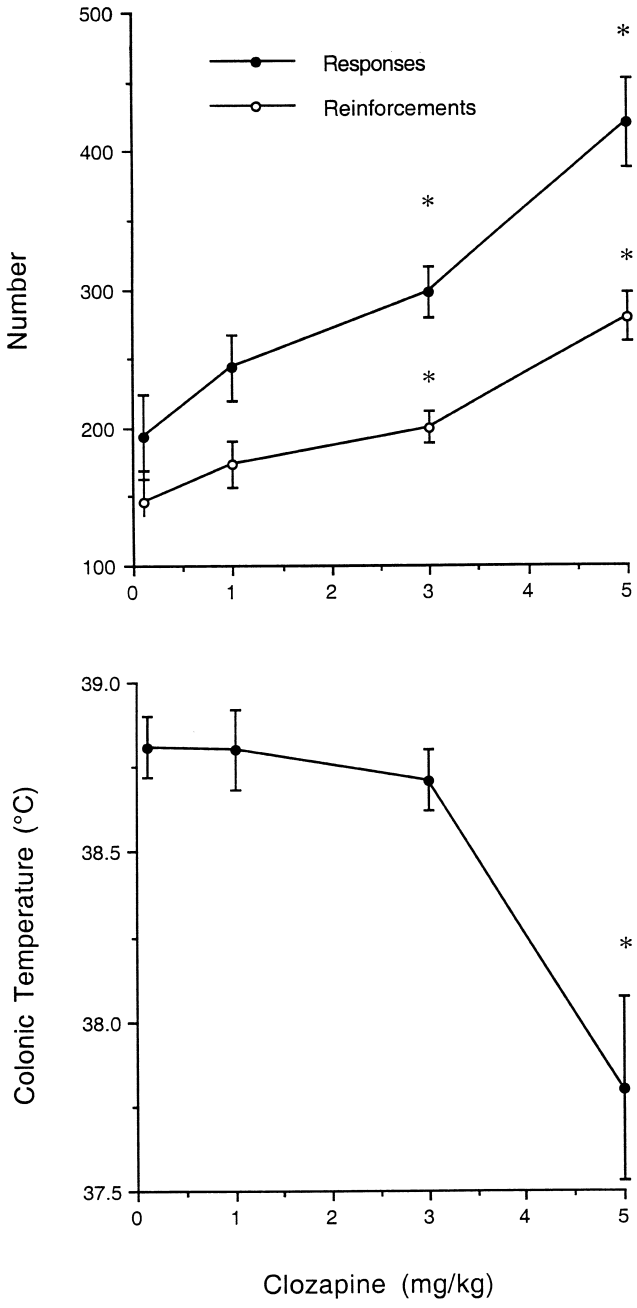

Fig. 1. Mean number of responses and reinforcements (±SEM) in the leverpress apparatus (3 s FDR schedule) and colonic temperature after vehicle and CLZ (1, 3 and 5 mg/kg). * $p<0.05$ (Fisher's PLSD test).

1.8. Data analysis

The primary data in Experiments 1 and 2 are the number of responses and reinforcements obtained in the leverpress apparatus for each dose of drug, and the change in T_c resulting from the treatments. Repeated measures ANOVAs were used for these experiments. Fisher's PLSD test was used for specific comparisons, with a significance level of 0.05.

The primary data in Experiment 3 are the frequency and duration of heat lamp activation and the change in T_c resulting from treatment. Paired *t*-tests were used for specific comparisons of CLZ to vehicle. Since a drug-induced change in the amount of heat received could be due to a change in the frequency of responding (responses per minute) or the duration of a response (second of heat per response), these values were also calculated.

The primary data in Experiment 4 are the effects of saline and CLZ on the position of the animal within the gradient (preferred T_a), and the change in T_c resulting from the treatments. A repeated measures ANOVA was used to test the overall significance of the treatments. Tukey's HSD test was used for multiple comparisons. Paired *t*-tests were used for comparing T_c pre-injection and post-test. All probabilities are two-tailed.

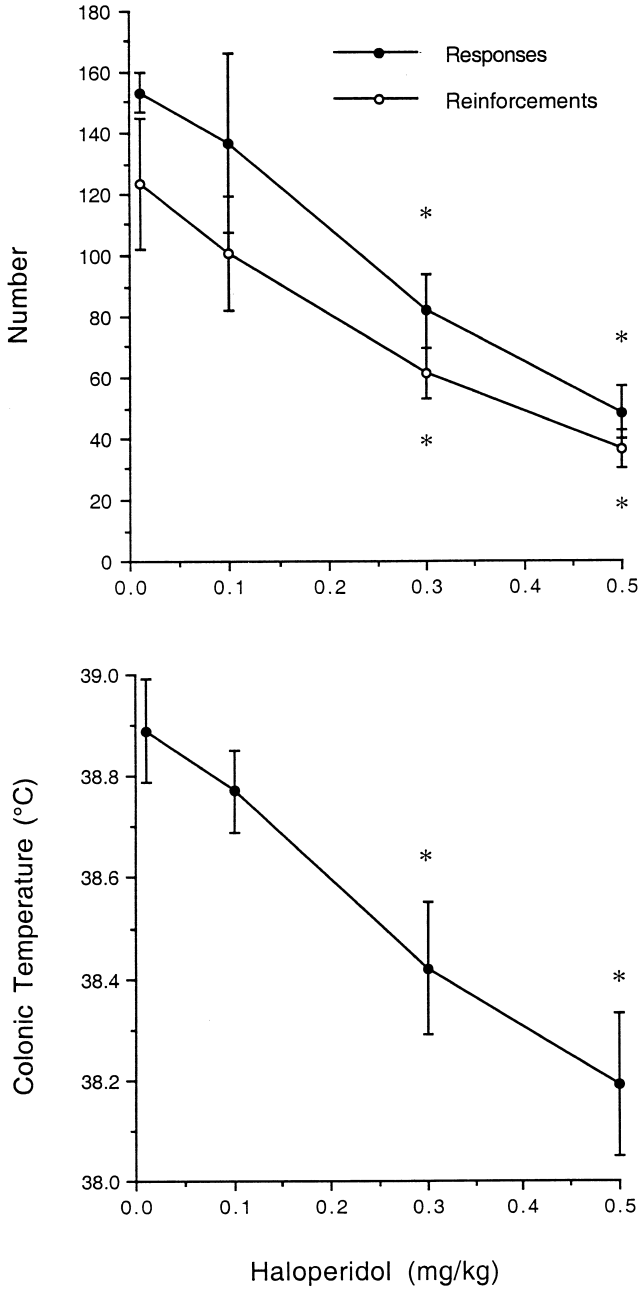

Fig. 2. Mean number of responses and reinforcements (±SEM) in the leverpress apparatus (3 s FDR schedule) and colonic temperature after vehicle and HAL (0.1, 0.3 and 0.5 mg/kg). * $p<0.05$ (Fisher's PLSD test).

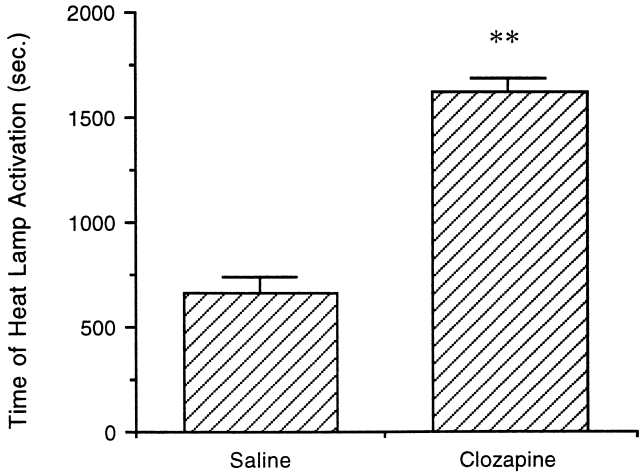

Fig. 3. Total duration of heat lamp activation (ad libitum reinforcement schedule) following saline and CLZ. ** $lp<0.01$ (paired *t*-test).

2. Results

2.1. Experiment 1

Fig. 1 shows the effects of CLZ on the number of responses and reinforcements in the leverpress apparatus (3 s FDR), and on T_c upon completion of the test. The number of responses and reinforcements increased as the dose of CLZ increased. There was a significant effect of drug

Table 1
Pre-injection and post-test T_c (°C) in the leverpress and thermal gradient

	Leverpress		Gradient	
	Pre-injection	Post-test	Pre-injection	Post-test
Saline	38.7 (±0.09)	38.7 (±0.07)	37.9 (±0.14)	37.9 (±0.17)
CLZ	38.7 (±0.09)	37.2 (±0.16)†	37.8 (±0.17)	38.5 (±0.24)†

Values are means (±SEM); $N=10$.
† $p<0.01$ (paired *t*-test).

treatment for numbers of responses ($F(3,24) = 13.1$, $p < 0.001$) and reinforcements ($F(3,24) = 15.0$, $p < 0.001$). The highest dose of CLZ also caused a significant decrease in final T_c ($F(3,24) = 12.2$, $p < .001$). Final mean T_c decreased to 37.8°C (5 mg/kg CLZ) from 38.8°C (vehicle). Responses to saline and vehicle were identical (data not shown).

2.2. Experiment 2

Fig. 2 shows the effects of HAL on the number of responses and reinforcements in the leverpress apparatus (3 s FDR) and on T_c upon completion of the test. Increasing doses of HAL significantly decreased the number of responses ($F(3,27) = 7.55$, $p < 0.001$), the number of reinforcements obtained ($F(3,27) = 9.44$, $p < 0.001$) and final T_c ($F(3,27) = 11.87$, $p < 0.0001$). The final mean T_c was 38.9°C for vehicle and 38.2°C for 0.5 mg/kg of HAL.

2.3. Experiment 3

Fig. 3 shows the effects of the highest dose of CLZ (5 mg/kg) and saline on the demand for heat in the leverpress apparatus when heat was available ad libitum. CLZ produced a significant increase in the behavioral demand for heat (243% greater than controls). However, the total amount of heat lamp activation for the CLZ group was insufficient to maintain their pre-injection T_c, which resulted in a significantly lower post-test T_c of 37.2°C compared to vehicle (Table 1). CLZ had an insignificant effect on the frequency of responding, increasing the rate of responding from 1.13 responses/min (saline) to 1.67. The duration of a response, however, increased significantly from 9.78 s/response (saline) to 16.54 ($t(9) = 7.8$, $p < 0.01$).

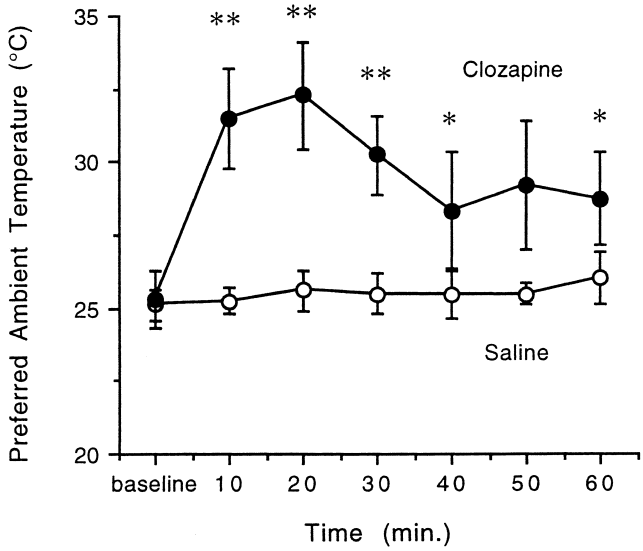

Fig. 4. Ambient temperature preference (°C) following saline and CLZ. * $p < 0.05$, ** $p < 0.01$ (Tukey's HSD test).

2.4. Experiment 4

Fig. 4 shows the effects of saline and CLZ on the preferred T_a in the gradient. Pre-injection T_c averaged 37.8°C (±0.17°C). All values of T_c are shown in Table 1. The ANOVA was significant for drug treatment ($F(5,90) = 58.3$, $p < 0.01$), time ($F(5,90) = 3.04$, $p < 0.05$) and the drug × time interaction ($F(5,90) = 4.14$, $p < 0.01$). CLZ produced a significant increase in preferred T_a in the gradient for every 10-min period following injection except during the 30–40-min interval. The overall preferred mean temperature was 25.6°C for saline and 30.1°C for CLZ. This resulted in a significantly higher post-test T_c compared to saline (Table 1).

3. Discussion

CLZ is known as an atypical antipsychotic due to the fact that it is not associated with serious deficits in motor functioning. The present study supports this idea. Compared to HAL, which induced a decrease in leverpress responding that corresponded to a decrease in T_c, CLZ induced a dose-dependent increase in leverpress responding, and this compensation was sufficient to keep T_c at control levels except at the highest dose tested. Even when given access to heat ad libitum, animals given a hypothermia-inducing dose of CLZ were unable to keep themselves warm despite increased responding. In the gradient test paradigm, treatment with CLZ produced significantly higher preferred T_a values, and this behavior allowed the animals to become hyperthermic relative to saline. The increased leverpress responding, coupled with the fact that the animals thermoregulate when heat access is easy, suggests the presence of a normal motivational component. It appears as though the amount of warmth made available to the animals by the heat lamps, even when they would stay on as long as the lever was held down, was simply not enough to overcome the powerful hypothermic effect of CLZ.

The opposing results obtained for HAL vs. CLZ suggest that, indeed, CLZ has very minimal (if any) effects on motor functioning. The fact that the gradient requires the least amount of motor effort does not appear to be as important as the fact that the gradient provides an environment warm enough for the animals to compensate for the effects of CLZ, while the leverpress does not.

These findings are supported by many other studies. In particular, Fowler et al. [5] and Fowler and Liou [6] have found that CLZ does not produce microcatalepsy and other motor deficits that interfere with operant responding for water, while typical neuroleptics such as HAL do. The question now becomes: By what mechanism does CLZ exert its hypothermic effects? It is clear that CLZ increases thermal conductance, most likely via vasodilatation. Past work has demonstrated that CLZ-induced hypothermia can be completely reversed by co-administration of D_1 or D_3

antagonists, suggesting that CLZ acts as an agonist at these receptor sites [12,13]. In fact, some have argued that this temperature-reducing effect plays a key role in CLZ's superior antipsychotic efficacy [7]. It would be interesting to examine the effects of the D_1 and D_2 antagonists that have been shown [12,13] to reverse CLZ-induced hypothermia to determine if they would produce different effects on operant responding.

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