Holtzman and Harlan Sprague-Dawley Rats: Differences in DRL 72-Sec Performance and 8-Hydroxy-Di-Propylamino Tetralin-Induced Hypothermia

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ABSTRACT
Several compounds were tested on the differential-reinforcement-of-low-rate 72-sec (DRL 72-sec) schedule, a behavioral screen to determine putative antidepressants; these compounds were evaluated in two outbred stocks of rats, Harlan and Holtzman Sprague-Dawley rats. A dose-response determination for the tricyclic antidepressants, imipramine and desipramine, the selective serotonin (5-hydroxytryptamine; 5-HT) reuptake inhibitor, fluoxetine, the 5-HT2 receptor antagonist, ketanserin, the 5-HT1A receptor agonist, (±)-8-hydroxy-di-propylamino tetralin (8-OH-DPAT) and the dopamine releasing compound, amphetamine, were assessed in both rat stocks. The two stocks of rats differed in their baseline performance on the DRL 72-sec schedule. The Harlan rats had a higher reinforcement rate and a lower response rate than the Holtzman rats. In Holtzman, but not in Harlan rats, imipramine, ketanserin, fluoxetine and 8-OH-DPAT increased reinforcement rate and decreased response rate on the DRL 72-sec schedule, confirming previous studies. However, desipramine was the only drug to increase reinforcement rate and decrease response rate in both Holtzman and Harlan rats; in Harlan rats, drugs that primarily act upon the 5-HT system, imipramine, ketanserin, fluoxetine and 8-OH-DPAT, disrupted the DRL 72-sec performance and did not increase the number of reinforcements over baseline as was seen in Holtzman rats. Amphetamine disrupted DRL 72-sec performance in both Holtzman and Harlan rats in a similar manner. The hypothermic response to 8-OH-DPAT was also assessed in the two stocks of rats; Holtzman rats had a smaller decrease in core body temperature than Harlan rats. The observed behavioral and pharmacological differences between Holtzman and Harlan rat stocks may be genetically and/or environmentally mediated.

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The differential reinforcement of low rate (DRL) 72-sec schedule of reinforcement has been shown to be a sensitive and specific tool for screening drugs with antidepressant-like activity (Marek et al., 1989a, b). The DRL 72-sec schedule is a lever pressing task that reinforces responses separated by at least 72 sec (see Ferster and Skinner, 1957; Sidman, 1955). Under this contingency the rats must wait at least 72 sec (IRT 72-sec) before pressing the lever to obtain water reinforcement; pressing the lever with an IRT that is less than 72 sec resets the 72-sec waiting period. Administration of antidepressants results in a characteristic change in the temporal distribution of responding such that the reinforcement rate increases and the response rate decreases or remains unchanged (McGuire and Seiden, 1980a, b; O'Donnell and Seiden, 1983; Richards et al., 1993a; Richards and Seiden, 1991). In addition, analysis of the IRT data indicate that antidepressants produce a coherent shift in the peak of the IRT relative frequency distribution toward longer IRTs (O'Donnell and Seiden, 1983; Richards and Seiden, 1991; Richards et al., 1993b). From a behavioral point of view the antidepressant drugs seem to affect the rat's ability to wait, which could be related to timing, memory, impulse control or degree of deprivation (Ho et al., 1996; Bizot et al., 1988; Evenden and Ryan, 1996). One, or a combination of these functions, could be a significant underlying physiological construct accounting for the change in the behavior of the rat.

The DRL 72-sec schedule has been a useful test for the screening of antidepressant drugs, and has helped to determine sites of antidepressant drug action in the central nervous system (Marek et al., 1989a, b; Dunn et al., 1993). Drugs that cause an antidepressant-like effect on the DRL 72-sec schedule include tricyclic antidepressants (O'Donnell and Seiden, 1983), monoamine oxidase inhibitors, the specific selective serotonin (5-HT) reuptake inhibitor, fluoxetine, (O'Donnell and Seiden, 1982; Marek et al., 1989a) and sev-

ABBREVIATIONS: 5-HT, 5-hydroxytryptamine; 8-OH-DPAT, (±)-8-hydroxy-di-propylamino tetralin; DRL, differential-reinforcement-of-low-rate; IRT, interresponse time; PkA, peak area; PkL, peak location.
eral atypical antidepressants (Marek et al., 1989b; Li et al., 1990). The antidepressant-like behavioral response on the DRL 72-sec schedule seems to depend on the noradrenergic and/or serotonergic systems in brain as indicated by pharmacological interactions with different receptor subtypes (Dunn et al., 1993; O'Donnell and Seiden, 1984; Seiden et al., 1987). Drugs from different classes, including psychomotor stimulants, opiates (morphine), anxiolytics, ethanol and antipsychotics do not engender the antidepressant-like pattern of responding on the DRL 72-sec schedule (O'Donnell and Seiden, 1983; Marek et al., 1989b; Marek and Seiden, 1988b; Jolly et al., 1998). Electroconvulsive shock also produces an antidepressant-like effect in rats trained on the DRL 72-sec schedule (Seiden et al., 1985).

Whether or not the DRL 72-sec schedule is a valid animal model of depression remains undetermined. However, as will be evidenced in comparing the two outbred stocks of rats used in this study, the DRL 72-sec schedule may have more value than its traditional role as a screen for antidepressants since one stock of rats (Harlan) obtained more reinforcers under drug-free conditions than the other stock (Holtzman). In addition to baseline differences, the two rat stocks had differential responses to several drugs. In general, drugs that act primarily upon the 5-HT system had an antidepressant-like effect in the Holtzman rats, but did not have a similar effect in the Harlan rats. Desipramine, which is a blocker of the norepinephrine transporter, had similar antidepressant-like effects on both stocks. Amphetamine disrupted responding, and the disruption was similar in both stocks of rats. Finally, the two stocks differed in their hypothalamic response to the 5-HT1A receptor agonist 8-OH-DPAT.

Materials and Methods

DRL 72-Sec Studies

Subjects. Two separate groups of rats were used. The first group consisted of 10 male Harlan Sprague-Dawley rats from Harlan Co. (Indianapolis, IN; Sprague-Dawley, SD) and 10 male Holtzman Sprague-Dawley rats from Holtzman Co. (Madison, WI; Sprague Dawley, SD). A second group consisted of 13 male Harlan rats and 15 male Holtzman rats (see drug administration). All rats weighed between 300 to 350 g and were housed two per cage in hanging stainless-steel wire cages. The lights in the colony room were on from 7:00 A.M. to 7:00 P.M. Food (4% Teklad rat food) was available ad libitum. Access to water was restricted to the amount of water obtained in the training or test session plus 20 min/day of free access to water at the end of each session. All rats were treated according to the standards set by the NIH.

Apparatus. Sixteen operant chambers were used. Each operant chamber was 20.5 cm wide, 20.5 cm deep and 23.5 cm long. The operant chambers had grid floors, aluminum front and back walls and Plexiglas sides. A lever was mounted 3 cm above the grid floor and 4.5 cm from the nearest side. A downward force of approximately 0.15 N was required to operate the lever. A solenoid-operated dipper was located 10 cm to the left of the lever. Access to the dipper was through a round 4.5 cm diameter hole in the front panel. When a lever press was reinforced, the dipper (0.025 ml) was lifted from a water trough to within reach of the rat's tongue for a period of 4 sec. The operant chambers were enclosed in sound attenuated chambers. The operant chambers were connected to a PDP-11/73 microcomputer via a Coulbourn Lablinic interface. The schedule contingencies were programmed and recorded using SRED-11 software system described by Snapper and others (Snapper et al., 1976).

Training. Upon arrival into the laboratory, the rats were adapted to the colony and to the 20 min/day water restriction regimen for 1 wk. The rats were trained to press a lever for water reinforcement in overnight training sessions using an alternate fixed-ratio one, fixed time 5-min schedule. Rats that failed to acquire the lever press response after five training sessions were shaped by the experimenter. After acquisition of the lever press response all further training occurred in daily (5 days/wk) 60-min sessions. The rats were then trained on a DRL 18-sec schedule of reinforcement for 3 wk. Finally, the rats were trained on the DRL 72-sec schedule of reinforcement for 8 wk before drug administration was initiated. A DRL 72-sec schedule specifies that only responses that result in interresponse times that are longer than 72 sec are followed by reinforcement.

Drug administration. The first group of rats (10 from Harlan and 10 from Holtzman) were tested with imipramine (2.5, 5, 10 mg/kg, i.p.; Sigma) and ketanserin (1, 3, 10 mg/kg, i.p.; RBI). The second group of rats (13 from Harlan and 15 from Holtzman) were tested with desipramine (1.25, 2.5, 5 and 10 mg/kg, i.p.; Sigma), fluoxetine (5, 10 and 20 mg/kg, i.p.; gift from Eli Lilly), amphetamine (1 and 1.5 mg/kg, i.p.; Sigma) and 8-OH-DPAT (0.05, 0.1 and 0.2 mg/kg, s.c.; RBI). Drugs were administered in a volume of 1 ml/kg body wt. 1-hr before testing with the exception of amphetamine and 8-OH-DPAT (no pretreatment time). Doses were calculated and expressed in terms of the salt. Ketanserin and fluoxetine were dissolved in distilled water and all other drugs were dissolved in 0.9% saline. All drug doses, including vehicle, were injected on Tuesday and Friday.

Quantitative analysis of interresponse times. An IRT is the elapsed time duration between two consecutive lever press responses. The effects of drug treatment on the IRT distributions generated by the DRL 72-sec schedule were quantitatively characterized using peak deviation analysis (Richards and Seiden, 1991; Richards et al., 1993b). Peak deviation analysis provides several measures of the shape of the IRT distribution including PkA and PkL. Burst responding was not systematically examined in the current work. An exponential curve predicts the appearance of the IRT histogram had the rat randomly emitted the same number of responses at the same overall rate. PkA is the area of the region above the hypothetical, exponential curve. PkL is the median location of the PkA. The PkA and PkL are used to characterize coherent shifts of an IRT distribution, but the shift itself is quantified by changes in PkA whose performance can be quantified by changes in PkA. A decrease in PkA is indicative of performance disruption, and no change in the PkA indicates a coherent shift when accompanied by a change in PkL. A drug has an “antidepressant-like” effect when there is an increase in the reinforcement rate; in most cases, the increase in reinforcement rate is accompanied by an increase in the PkL, with little or no change in PkA.

Data analysis. Response rate, reinforcement rate, PkA and PkL were measured for each rat for each treatment. At 0.1 and 0.2 mg/kg 8-OH-DPAT none of the Harlan rats obtained the minimum number of responses, and therefore there were no PkL and PkA data available at these doses. Baseline values of DRL 72-sec performance were obtained for each group by combining the data of all the Thursdays (drug-free day) for each dose-response determination.

Data are presented as mean ± S.E.M. A one-way repeated measures analysis of variance was done for each dose-response function on each of the dependent variables (response rate, reinforcement rate, PkL and PkA; for details see Richards et al., 1993b). After a significant analysis of variance, post hoc multiple comparisons were performed using the Dunnett test (Winer, 1971). Baseline differences between Holtzman and Harlan rats were calculated by combining the animals from the two sets of rats used throughout the study. These data were analyzed using a Student’s t test (Winer, 1971). The alpha value (two-tailed) required for significance was set at 0.05. All statistics were done on the absolute numbers of responses, reinforcements, PkL and PkA, but the data are reported for the sake of clarity as percentage change from vehicle.
Hypothemic Response to 8-OH-DPAT

**Subjects.** Eight male Harlan and eight male Holtzman rats were housed under identical conditions as described above except that both food and water were available *ad libitum*.

**Temperature measurement apparatus.** For this experiment, novel temperature measurement chambers were used as described by Malberg and Seiden (1997). This system maintains a constant ambient temperature (24 ± 0.5°C in the present experiment) and measures the rat’s core body temperature in a noninvasive manner by radio telemetry.

The temperature chambers were modified refrigerators that have, in addition to a compressor to cool the chamber, a thermistor to record ambient temperature, a strip heater, and a fan to ensure that the environmental temperature is constant. Inside the chamber is a cage (20.3 cm wide, 15.2 cm high and 16.5 cm deep) where the rat is placed. To measure core body temperature, a temperature-sensitive transmitter (Mini-mitter, Sun River, OR) that was previously implanted into the peritoneal cavity of the rat emits a signal proportional to the core body temperature. The system is controlled with a standard microcomputer that has an analog-to-digital interface, and the interface allows the environmental and core body temperatures to be recorded. Both core body temperature and ambient temperature readings were recorded once a second and then averaged to produce one reading per minute.

**Surgery.** The core body temperature transmitters were implanted in anesthetized rats [ketamine (1 ml/kg) and xylazine (0.33 ml/kg)]. A midline cut was made in the peritoneum and a sterilized transmitter was inserted into the peritoneal cavity as described by Farfel and Seiden (1995). Rats were given a minimum of 3 days to recover from the surgeries before experimental drug injections.

**Test procedure.** Rats were acclimated to the temperature chambers for 1 hr before the injection of the drug. After administration, core body temperature was continuously recorded for a 2-hr period. The test was performed each day for 4 consecutive days, one dose a day, including saline.

**Drug administration.** Both stocks of rats were tested with 8-OH-DPAT (0.1, 0.3 and 1 mg/kg, s.c.).

**Data analysis.** Maximal core body temperature drop for each dose of 8-OH-DPAT was calculated for each rat. The maximal drop in core body temperature was calculated by subtracting the minimal core body temperature value from the maximal core body temperature value for each individual rat. These maximal drop values were averaged for each animal at each dose. Differences between stocks were determined with the Student’s t test (Winer, 1971) at each dose. The alpha value (two-tail) required for significance was 0.05.

Results

**DRL 72-sec Schedule**

**Baseline performance.** The performance of Harlan and Holtzman rat reached stability on the DRL 72-sec schedule after 8 wk of training. The two stocks of rats differed in their baseline performance (fig. 1). The two stocks of rats differed in their baseline performance (fig. 1). The Harlan rats had a lower response rate (Harlan: 59.6 ± 2.6; Holtzman: 88.2 ± 5.0) and a higher reinforcement rate (Harlan: 16.6 ± 0.9; Holtzman: 9.9 ± 0.7) than the Holtzman rats. These baseline differences were significant between groups (P < .001) and were maintained throughout the duration of the experiment. The control baseline data were obtained by combining the performance of every Thursday (neither drug nor saline was administered on these days) during the dose response determinations. The PkL of the Harlan rats was longer than the PkL of the Holtzman rats (Harlan: 66.8 ± 2.3; Holtzman: 55.1 ± 1.6), and the PkA was larger in the Harlan compared to the Holtzman rats (Harlan: 0.53 ± 0.02; Holtzman: 0.46 ± 0.01; fig. 1). It should be noted that these same differences were apparent between two separate batches of rats considered here and a third batch of each rat stock that was tested before the rats discussed in this experiment (data not shown); thus, the differences between the two stocks were a robust and consistent finding.

**Effects of imipramine.** For the Holtzman rats, imipramine increased the reinforcement rate [F(4,36) = 8.7, P < .01] and decreased the response rate [F(4,36) = 10.3, P < .0001] in a dose-dependent fashion. There was an increase in PkL [F(4,36) = 7.7, P = .01] and in PkA [F(4,36) = 2.7, P = .05]. Imipramine had an antidepressant-like effect in the Holtzman rats. In contrast, for the Harlan rats, there was not a significant increase in reinforcement rate [F(4,40) = 0.8, P = .50]. The response rate was significantly decreased [F(4,40) = 15.4, P < .0001], PkL [F(4,40) = 1.1, P = .40] and PkA [F(4,40) = 1.2, P = .34] was not significantly changed from baseline. Imipramine did not have an antidepressant-like effect in Harlan rats (fig. 2).
Effects of ketanserin. For the Holtzman rats, ketanserin significantly increased the reinforcement rate $[F(4,36) = 22.3, P < .01]$ and decreased the response rate $[F(4,36) = 8.1, P < .0001]$ in a dose-dependent fashion. There was an increase in PkL $[F(4,36) = 8.9, P < .0001]$, but PkA was not significantly changed $[F(4,36) = 1.2, P = .36]$. Ketanserin had an antidepressant-like effect in Holtzman rats. In contrast, for the Harlan rats, there was not a significant increase in reinforcement rate $[F(4,36) = 2.4, P = .07]$. The response rate was significantly decreased $[F(4,36) = 10.6, P < .0001]$, PkL was significantly increased $[F(4,36) = 9.1, P < .0001]$ and PkA was significantly decreased $[F(4,36) = 11.2, P < .01]$. Ketanserin did not have an antidepressant-like effect in Harlan rats (fig. 3).

Effects of fluoxetine. For the Holtzman rats, fluoxetine significantly increased the reinforcement rate $[F(4,56) = 23.1, P < .0001]$ and decreased the response rate $[F(4,56) = 24.9, P < .0001]$ in a dose-dependent fashion. There was an increase in PkL $[F(4,56) = 9.3, P < .0001]$. The overall PkA was significantly decreased $[F(4,56) = 8.8, P < .0001]$; the post hoc test showed no significant change at 1 and 5 mg/kg of fluoxetine but there was a significant reduction for 10 mg/kg of fluoxetine. Fluoxetine had an antidepressant-like effect in Holtzman rats. In contrast, for the Harlan rats, there was an overall decrease in the reinforcement rate $[F(4,48) = 3.1, P < .05]$; post hoc multiple comparisons did not identify a significant dose effect. The response rate was significantly decreased $[F(4,48) = 7.3, P < .0001]$, PkL was significantly increased $[F(4,48) = 5.6, P = .0008]$ and there were no significant changes in the PkA $[F(4,48) = 1.2, P = .31]$. Fluoxetine did not have an antidepressant-like effect in Harlan rats (fig. 4).

Effects of 8-OH-DPAT. For the Holtzman rats, 8-OH-DPAT significantly increased the reinforcement rate $[F(4,56) = 23.1, P < .0001]$ and decreased the response rate $[F(4,56) = 20.2, P < .0001]$ in a dose-dependent fashion. There were
changes in the PkL $F(4,56) = 2.7, \ P = .04$; post hoc multiple comparison did not identify a significant dose effect. There was a significant decrease in PkA $F(4,56) = 13.8, \ P < .0001$. The effects of 8-OH-DPAT on reinforcement rate and response rate in Holtzman rats were antidepressant-like; however the PkA was disrupted. In contrast for the Harlan rats, there was a significant decrease in the reinforcement rate $F(4,48) = 27.4, \ P < .0001$ and the response rate was significantly decreased $F(4,48) = 69.3, \ P < .0001$. There were no significant changes in PkL $F(2,24) = 0.4, \ P = .639$ and PkA $F(2,24) = 2.3, \ P = .13$. The 0.1- and 0.2-mg/kg doses were not valid values because all the rats made less than 25 responses per session. 8-OH-DPAT did not have an antidepressant-like effect in Harlan rats (fig. 5).

**Effects of desipramine.** For the Holtzman rats, desipramine significantly increased the reinforcement rate $F(5,70) = 19.52, \ P < .0001$ and decreased the response rate $F(5,70) = 13.2, \ P < .0001$ in a dose-dependent fashion. There was an increase in PkL $F(5,70) = 9.9, \ P < .0001$ and there were no significant changes in PkA $F(5,70) = 1.2, \ P = .33$. Desipramine had an antidepressant-like effect in Holtzman rats. Similarly, for the Harlan rats, desipramine significantly increased the reinforcement rate $F(5,60) = 8.8, \ P < .0001$ and decreased the response rate $F(5,60) = 9.9, \ P < .0001$ in a dose-dependent fashion. There was an increase in PkL $F(5,60) = 10.3, \ P < .0001$ and there were significant changes in PkA $F(5,60) = 3.4, \ P < .01$; post hoc multiple comparison did not identify a significant dose. Overall, desipramine had an antidepressant-like effect in Harlan rats (fig. 6).

**Effects of amphetamine.** For the Holtzman rats, amphetamine significantly decreased the reinforcement rate $F(3,42) = 4.1, \ P < .05$ and increased the response rate $F(3,42) = 9.4, \ P < .0001$. There was a significant decrease in PkL $F(3,42) = 74.6, \ P < .0001$ and PkA $F(3,42) = 63.2, \ P < .0001$. Similarly for the Harlan rats, amphetamine significantly decreased the reinforcement rate $F(3,36) = 34.3, \ P < .0001$ and increased the response rate $F(3,36) = 5.6, \ P < .05$. There was a significant decrease in PkL $F(3,36) = 28.3, \ P < .0001$ and PkA $F(3,36) = 65.4, \ P < .0001$. Amphetamine did not produce an antidepressant-like effect in either the Holtzman or Harlan rats, but amphetamine did...
produce response patterns typical for psychomotor stimulants in both stocks of rats (fig. 7).

Summary of drugs on DRL reinforcement rate. One of the most reliable and readily identifiable effects of antidepressants on DRL 72-sec schedule is an increase in reinforcement rate. In summary, the reinforcement rate of Holtzman rats was increased by imipramine, ketanserin, fluoxetine and 8-OH-DPAT; these drugs did not significantly affect the reinforcement rate of Harlan rats. Both Harlan and Holtzman rats had a decreased reinforcement rate and IRT distributions that were shifted toward shorter IRTs after amphetamine. Desipramine increased the reinforcement rate of both Harlan and Holtzman rats. As summarized in table 1, several 5-HT-acting drugs increased the reinforcement rate of only the Holtzman rats whereas both Harlan and Holtzman rats responded to amphetamine and desipramine.

Hypothermic Response to 8-OH-DPAT

Although there were no baseline temperature differences between the two stocks, administration of 8-OH-DPAT induced a dose-dependent decrease in core body temperature in both Holtzman and Harlan rats (fig. 8; top). The maximal drop in core body temperature was significantly more pronounced in Harlan rats at .1 mg/kg (P < .05), .3 mg/kg (P < .05) and 1 mg/kg (P < .01) 8-OH-DPAT (fig. 8; bottom).

Discussion

In our studies we report differences between Harlan and Holtzman rat stocks. There were baseline differences in the four variables that describe performance on the DRL 72-sec schedule, with the stocks of rats differing in response and reinforcement rate, PkL and PkA. The Harlan rats have a lower response rate than the Holtzman rats; reinforcement rate was higher in Harlan rats as compared to the Holtzman rats. The PkL was also longer in the Harlan compared to the Holtzman rats and the PkA was larger in the Harlan compared to Holtzman rats. Furthermore, according to the criteria for an antidepressant-like effect on the DRL 72-sec schedule, the Harlan rats exhibited a response pattern that is...
similar to Holtzman rats that had already been treated with an antidepressant drug. Because the spontaneous performance of the Harlan rats is consistently better than the Holtzman rats, we suggest that there may be an endogenous factor that differs between the Harlan and the Holtzman rats. Differences in the functional status of the 5-HT system were corroborated in the second part of the study using a temperature paradigm. The two stocks of rats were found to differ in their hypothermic response to the 5-HT1A agonist 8-OH-DPAT, with greater hypothermia occurring in the Harlan rats as compared to Holtzman rats.

In addition to baseline differences on the DRL 72-sec schedule, the two stocks of rats had different reinforcement patterns in response to some drugs that have their major pharmacological impact on the 5-HT system; these drugs include ketanserin, imipramine, 8-OH-DPAT and fluoxetine. Although both Harlan and Holtzman rats had decreases in response rate after administration of these drugs, only the Holtzman rats showed an increase in reinforcement rate. However, both stocks of rats had similar responses to the NE transporter blocker desipramine, and the DA releasing agent amphetamine. Differences in the responses to the various drugs between the two stocks of rats could be a function of the baseline rate of responding or baseline rate of reinforcement; this is the case if one considers the drugs that act on the 5-HT system. Under baseline conditions, the Holtzman rats have higher response rates and lower reinforcement rates compared to the Harlan rats and the change in the Holtzman rats in response to 5-HT acting drugs may be due to the fact they initially had comparatively poorer performance. However, although Harlan and Holtzman differed in baseline reinforcement rate, both stocks of rats showed increases in reinforcement rate when receiving desipramine, and showed similar response rate increases and reinforcement rate decreases in response to amphetamine. Thus, although desipramine and amphetamine had different effects upon reinforcement rate in the two stocks of rats, it is noteworthy that these effects were similar in Harlan and Holtzman rats. The similar behavioral response to desipramine and amphetamine in the two stocks of rats suggest that the differences in responding we observed with the 5-HT-acting drugs are not simply a function of differences in baseline responding. Additional
studies with other noradrenergic and dopaminergic drugs are being conducted.

The effects of particular drugs on DRL 72-sec performance warrants further consideration. The studies with 8-OH-DPAT in the Holtzman rats show ambiguity as to whether or not to call this an antidepressant-like effect. Even though reinforcements were increased, the PkL was not increased and the PkA was decreased by this drug; therefore, 8-OH-DPAT does not have all of the properties of other antidepressants that were tested in the DRL schedule. This class of 5-HT1A receptor agonist also has an anxiolytic profile (File et al., 1996; Gonzalez et al., 1996; Soubrie, 1989; Marek et al., 1989a; Sussman, 1994) in addition to its antidepressant effects, which may underlie the observed behavioral profile in the DRL schedule. This class of 5-HT1A receptor agonist also has an anxiolytic profile (File et al., 1996; Gonzalez et al., 1996; Soubrie, 1989; Marek et al., 1989a; Sussman, 1994) in addition to its antidepressant effects, which may underlie the observed behavioral profile in the DRL schedule. We also observed that at the highest dose of fluoxetine (20 mg/kg), the PkA was decreased despite the fact that the reinforcement rate was increased. Although it is typical of an antidepressant-like effect for there to be little or no change in the PkA, fluoxetine nevertheless had a full antidepressant-like effect at the 10-mg/kg dose, which is in accordance with a previous study (Richards et al., 1993a).

Drugs that affect the 5-HT system have previously been shown to have antidepressant-like actions in rats on the DRL 72-sec, as well as in other antidepressant screens (Seiden et al., 1987; Marek et al., 1989a; Stahl, 1993; Wieland and Lucki, 1990; Singh and Lucki, 1993; Overstreet et al., 1996). Furthermore, several studies have shown that depressed individuals have a blunted hypothermic response to 5-HT1A agonists when they are compared to nondepressed controls (Cowen et al., 1994; Lesch et al., 1990; but see Meltzer and Maes, 1995). Blunted hypothermic responses after 5-HT1A agonists in patients with depression appear to result from a 5-HT1A receptor-associated abnormality, thus supporting the hypothesis that altered serotonergic activity may be present in psychopathologically defined subtypes of affective disorder. Taking this into account, it is of interest that the Holtzman rats show a blunted hypothermic response when they are compared with the Harlan rats. Such hyporeactivity of 5-HT functions in the Holtzman stock raises the question...
of the relationship between the functional state of the serotonergic system and depression-like behaviors in experimental models of depression. Although this correspondence may be premature, it is of great interest due to the fact that the Harlan rats have superior performance on the DRL 72-sec screen for antidepressants when compared to the Holtzman rats. Furthermore, Holtzman rats improve their performance when serotonergic antidepressant drugs are administered.

In summary, Harlan and Holtzman rats have a different baseline DRL 72-sec schedule profile, respond on the DRL 72-sec schedule differently to serotonergic drugs and differ in the magnitude of hypothermia induced by 8-OH-DPAT, but respond similarly to those drugs acting upon the dopamine and norepinephrine systems.

In our study, environmental differences between Harlan and Holtzman rats were not present when the rats entered the laboratory because they were handled in an identical fashion. Nevertheless, important environmental differences may have been present before these rats were delivered to our laboratory. It is known that early environmental conditions can influence the development of the rat brain and behavior (Greenough et al., 1986; Sudakov et al., 1996). The rearing of the Harlan rats at the site in Indiana is similar to the rearing of the Holtzman rats at the site in Wisconsin.

**Fig. 7.** Amphetamine dose response determination. Harlan rats (n = 13); Holtzman rats (n = 15). Vehicle values given as Holtzman and Harlan, respectively (S.E.M.). Response rate: 98.9 (8.1), 66.4 (2.3); reinforcement rate: 7.9 (1.3), 15.8 (1.2); PkL: 51.8 (2.9), 63.5 (2.0); PkA: 0.46 (0.02), 0.54 (0.02).

**TABLE 1**
Summary table showing the effects of several drugs upon the DRL 72-sec reinforcement rate of Harlan and Holtzman rats

<table>
<thead>
<tr>
<th>DRUG (dose)</th>
<th>Reinforcement Rate</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Harlan</td>
</tr>
<tr>
<td>8-OH-DPAT (0.05, 0.1, 0.2 mg/kg, sc)</td>
<td>↓↓</td>
</tr>
<tr>
<td>Imipramine (2.5, 5.0, 10.0 mg/kg, ip)</td>
<td>=</td>
</tr>
<tr>
<td>Ketanserin (1.0, 3.0, 10.0 mg/kg, ip)</td>
<td>=</td>
</tr>
<tr>
<td>Fluoxetine (5.0, 10.0, 20.0 mg/kg, ip)</td>
<td>=</td>
</tr>
<tr>
<td>Desipramine (1.25, 2.5, 5.0, 10.0 mg/kg, ip)</td>
<td>↑↓↑</td>
</tr>
<tr>
<td>Amphetamine (1.0 and 1.5 mg/kg, ip)</td>
<td>↓↓</td>
</tr>
</tbody>
</table>

The doses of each drug are listed; the number of "↑" or "↓" symbols indicates the number of doses in which the reinforcement rate increased or decreased, respectively. "= = " indicates that no significant effect of the drug was observed at any dose.
However, different laboratory feed is used at the two facilities (Harlan Sprague-Dawley, personal communication) and it is possible that this could account for the observed differences between these two stocks of rats. Whether the differences between the two stocks of rats may be due to differences in the density and/or affinity of the 5-HT1A receptor (Hulihan-Giblin et al., 1992), differences in the nucleotide sequence of the 5-HT1A receptor DNA, or may also be related to the activity of second messengers coupled to the 5-HT1A receptor, possibilities that we are currently exploring. Although it is possible that rearing conditions produced the differences between Harlan and Holtzman rats, other studies suggest differences between stocks of rats can occur even when rearing conditions are similar. Thus, this laboratory is exploring the possibility that the underlying substrate of the presently observed differences between the Harlan and Holtzman rats is at the genomic level or is due to different environmental conditions. At the very least these results suggest that care must be exerted when comparing results using rats obtained from different suppliers (Overstreet and Rezvani, 1996; Pare and Kluczynski, 1997).

In summary, we observed baseline behavioral differences between Harlan and Holtzman rats performing on the DRL 72-sec schedule; in addition, we observed pharmacological differences between the two stocks of rats on the DRL 72-sec schedule. These pharmacological differences suggest that the endogenous differences may reside in the 5-HT, but not NE or DA systems. Interestingly, we also showed that the Holtzman rat stock with the poorest performance on the DRL 72-sec schedule showed a blunted hypothermic response to the 5-HT1A receptor agonist, 8-OH-DPAT. We therefore speculate that the genetically and/or environmentally induced 5-HT1A abnormalities may underlie the presently observed differences between Harlan and Holtzman rats.

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References


Fig. 8. 8-OH-DPAT-induced hypothermia in Harlan and Holtzman rats plotted against time (top) and the maximal drop in core temperature elicited by 8-OH-DPAT in the two rat stocks (bottom).

However, different laboratory feed is used at the two facilities (Harlan Sprague-Dawley, personal communication) and it is possible that this could account for the observed differences between these two stocks of rats. Whether the differences are genetically or environmentally induced remains a subject for future experiments. However, rats selectively-bred and reared under identical environmental conditions were also found to differ in magnitude of 8-OH-DPAT hypothermia (Overstreet et al., 1996) in a manner similar to our study. Focusing at the genomic level, because we have shown that the Harlan and Holtzman rats responded differentially to hypothermia engendered by the 5-HT1A receptor agonist 8-OH-DPAT, we are attempting to sequence the 5-HT1A receptor gene of both Harlan and Holtzman rats. Our focus on the 5-HT1A receptor gene is the starting point in these experiments, although other genes or polymorphism may be involved (Berrettini et al., 1990, 1997). The differences between the two stocks of rats may be due to differences in the density and/or affinity of the 5-HT1A receptor (Hulihan-Giblin et al., 1992), differences in the nucleotide sequence of the 5-HT1A receptor DNA, or may also be related to the activity of second messengers coupled to the 5-HT1A receptor, possibilities that we are currently exploring. Although it is possible that rearing conditions produced the differences between Harlan and Holtzman rats, other studies suggest differences between stocks of rats can occur even when rearing conditions are similar. Thus, this laboratory is exploring the possibility that the underlying substrate of the presently observed differences between the Harlan and Holtzman rats is at the genomic level or is due to different environmental conditions. At the very least these results suggest that care must be exerted when comparing results using rats obtained from different suppliers (Overstreet and Rezvani, 1996; Pare and Kluczynski, 1997).

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References


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