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LSD Produces Conditioned Place Preference in Male But Not Female Fawn Hooded Rats

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MEEHAN, S. M. AND M. D. SCHECHTER. *LSD produces conditioned place preference in male but not female fawn hooded rats.* PHARMACOL BIOCHEM BEHAV **59**(1) 105–108 1998.—Male and female Fawn Hooded rats were examined for conditioned place preference (CPP) or aversion (CPA) to lysergic acid diethylamide (LSD). Using a biased design, experimental animals were trained with LSD (0.2 mg/kg, IP) administered in conjunction with confinement in either the preferred or nonpreferred location. Control animals received confinement in both locations after administration of saline. Results indicated that rats administered LSD while sequestered in the nonpreferred location spent more time in that location during a nondrug test. This effect, indicative of a conditioned place preference, was exhibited only in male animals. Results are discussed in terms of potential sex differences that may mediate serotonergic sensitivity in the Fawn Hooded rat strain. © 1998 Elsevier Science Inc.

Fawn Hooded rats LSD Conditioned place preference Sex differences

THE Fawn Hooded (FH) strain of rat possesses a genetic platelet storage pool deficiency resulting in a marked impairment in the ability of platelets to store and release serotonin (5-HT) (18). The relationship between this blood abnormality and alterations in brain levels of 5-HT is unclear. However, neurochemical studies have shown reduced levels of brain 5-HT in FH rats compared to other strains (8). Evidence gathered using pharmacological interventions has also indicated that the FH rat strain is differentially sensitive to the actions of numerous serotonergic agonists (1,2,10,23). Furthermore, behavioral studies also have determined that the FH rat displays differential responding to 5-HT agonists, for example, fenfluramine has been shown to produce a conditioned place preference in FH rats but not in Sprague–Dawley rats (16). Data also indicate that male and female FH rats exhibit differential response patterns in training with MDMA as a discriminative cue (20). This sex difference in drug sensitivity in the FH rat has also been observed with ethanol in that male FH rats appear be more sensitive to its intoxicating effects (11).

A recent study by Parker (17) reported that the serotonergically active agent lysergic acid diethylamide (LSD, 0.2 mg/kg) produced a conditioned place preference for a distinctive location that had been paired with drug administration in male Sprague–Dawley rats. The purpose of the present study was to extend this work by examining the production of both a conditioned place preference and/or aversion to LSD (0.2 mg/kg) in both male and female FH rats. This latter result will evidence potential sex differences in 5-HT sensitivity within the FH strain.

METHOD

Subjects

Thirty male and 30 female Fawn Hooded rats were employed as subjects. Animals were born and raised in the vivarium facility at NEOUCOM from parent stock obtained from the New York State Department of Health. Rats were 60–90 days of age at the time of experimentation and were housed individually in suspended wire cages in a climate controlled colony room. Animals were maintained on a 12 L:12 D cycle with light onset occurring at 0600 h. Food and water were available ad lib.

Apparatus

The apparatus consisted of four stainless steel units (Lafayette Instrument Co., Lafayette, IN, Model #85000) each divided into three distinctive chambers. The "white" chamber

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was 30 × 20.5 × 18 cm and illuminated by a 9 W lightbulb fixed directly above a translucent white Plexiglas lid. The floor of this chamber consisted of stainless steel rods with clean aspen shavings placed beneath. The "black" chamber was of the same dimensions and equipped with a duplicate light source. However, red plastic placed between the bulb and the lid allowed for red illumination within the chamber. The floor on this side was covered with black smooth Plexiglas. The third, middle, chamber consisted of an open ended gray-colored stainless steel box and was nonremarkable. This box could be isolated from the black and white chambers during training by insertion of metal plates (Lafayette Model #85009) or left open to allow unrestricted locomotion between the black and white sides during testing. Entry into either the white or black side closed a microswitch which, in turn, started a timer and, thus, the time (in seconds) that a rat spent in each of the three chambers was recorded by a computer.

Procedure

The procedure followed a standard cycle across consecutive days. The cycle consisted of habituation to the training room, free access to the apparatus, and baseline preference measurement, drug or vehicle injection combined with place conditioning over eight sessions, and last, the test of place preference without drug administration.

Habituation occurred on days 1 and 2. Animals remained in their home cages and were transferred from the colony room to the training room where they remained for 1 h each day. During this time each animal was handled for 2–3 min. On days 3 and 4, each animal was given 15 min of free access to the entire conditioned place preference apparatus with the center chamber opened to allow free entry into both the black and white sides. The apparatus was cleaned after each exposure to preclude olfactory cues. On day 5, animals were placed into the center chamber of the apparatus and the time spent (in seconds) in each of the three chambers was monitored for 15 min; this constituted the baseline test. At this time, the side in which the rat spent the majority of its time was designated as its preferred side, whereas the side in which less time was spent was considered its nonpreferred side.

Place conditioning began on day 6 and continued through day 13. Animals were assigned to six equal groups contingent upon baseline black/white preference. Two control groups (n = 10 males, n = 10 females) were administered saline and confined to the nonpreferred and preferred side, alternating for 8 days. Two experimental groups (n = 10 males and n = 10 females) received an injection of LSD and were confined to their nonpreferred side, whereas on alternate days they received saline and were confined to their preferred side. One group of males and one group of females received LSD paired with their preferred side and saline paired with their nonpreferred side. Each experimental animal was given eight pairings, four with LSD and four with saline, beginning with the drug and alternating with saline on successive days. Each pairing consisted of an IP injection of 0.2 mg/kg LSD or saline given 10 min prior to confinement in the designated chamber. Animals remained confined in the chamber for 30 min and were then removed and returned to their home cage.

On day 14, testing was conducted in a manner identical to that used for the baseline preference test on day 5, in that animals were tested in the absence of the drug for 15 min of free access with their location in the apparatus automatically monitored and recorded in seconds.

RESULTS AND DISCUSSION

Time spent on the preferred and nonpreferred sides during baseline testing in the experimental and control groups was analyzed using separate between groups analysis of variance (ANOVA). Results for both males and females revealed no differences between the control and experimental groups in the time spent on the nonpreferred side, $F(2, 27) = 0.12$ and 0.15. Similarly, time spent on the preferred side during baseline tests was equivalent for male and female animals in the experimental and control groups, $F(2, 27) = 0.74$ and 0.189.

Postconditioning test data were analyzed using a 2 × 3 split plot ANOVA comparing baseline vs. test scores in the two experimental groups and the saline control group. Assessment of changes in time spent on the preferred side in males failed to yield a significant interaction [$F(2, 27) = 0.39$, NS]. Furthermore, there were no main effects of either test time (baseline vs. postconditioning test), $F(2, 27) = 0.40$, NS, or group, $F(1, 27) = 2.53$. Similar effects were also observed in females, $F(2, 27) = 0.28$ and 0.10, respectively; $F(1, 27) = 2.89$, (see Fig. 1). These results suggest that LSD administration in conjunction with confinement in the preferred side during training did not reduce preference for that side, i.e., LSD failed to produce a conditioned place aversion in male or female animals. Furthermore, these results indicate that mere exposure to the apparatus did not effect side preference as seen in the saline control rats.

Analysis of the time spent by males on the nonpreferred side also failed to reveal an interaction or group main effect, $F(2, 27) = 0.31$ and 0.11, respectively. However, there was a significant main effect of test time, $F(1, 27) = 8.26$, $p < 0.01$. Contrast effects analyses revealed that this result was produced by an increase in time spent on the nonpreferred side during testing in animals that had received LSD in conjunction with confinement in the nonpreferred side during training, $F(1, 27) = 4.93$, $p < 0.05$ (see Fig. 2). Animals that received LSD followed by placement in the preferred side, as well as animals in the saline control group, failed to show a similar shift in side

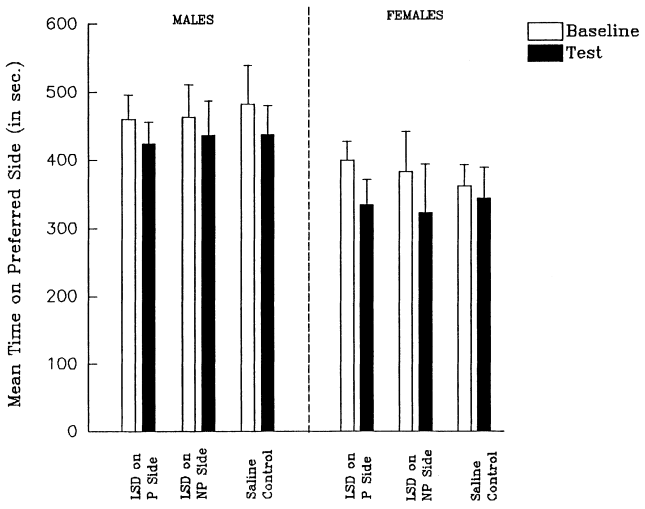

FIG. 1. Mean time (s) spent in the preferred side of the test chamber during baseline and postconditioning tests in male and female FH rats (n = 10 per group) that received IP 0.2 mg/kg LSD on the preferred (P) or nonpreferred (NP) sides, as well as those receiving saline on both sides.

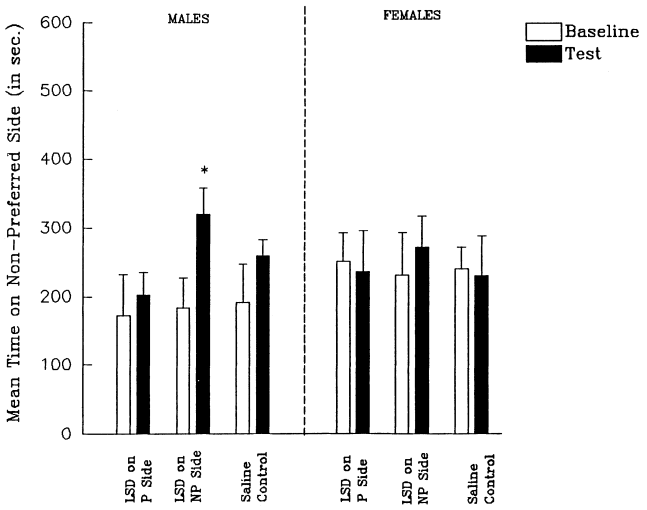

FIG. 2. Mean time (s) spent in the nonpreferred side during baseline and postconditioning tests in male and female FH rats (n = 10 per group) who were injected IP with 0.20 mg/kg LSD on the preferred (P) or nonpreferred (NP) sides or with saline on both sides. *$p < 0.05$, difference from time spent on the NP side during baseline test.

preference, $F(1, 27) = 3.26$ and 1.00, respectively. Analysis of time spent on the nonpreferred side by females produced no significant interaction or main effects (all F-values < 1.00). These results suggest that LSD presented in conjunction with confinement in a nonpreferred environment leads to an increase in the amount of time spent in that environment during testing, i.e., LSD produced a conditioned place preference in Fawn Hooded rats. However, this effect was observed only in males.

One possible factor that could account for the failure to observe an effect in females hinges on a methodological constraint inherent in the conditioning paradigm. Specifically, training was conducted over an extended period of time and stages of the estrus cycle were neither assessed nor controlled. Studies have indicated that estrogens are associated with decreased metabolism of serotonin in the rat CNS (21). Thus, it is at least plausible that the response to a drug may vary, contingent upon the estrus stage present at the time of exposure. In the current study, training and testing were not restricted to specific stages of the cycle, and thus occurred at random points within at least two consecutive cycles. This methodological constraint may have introduced a source of variability that obscured the conditioned drug effect in females. Future research examining place preference conditioning as a function of estrus cycle stage will be necessary to resolve this point.

Despite the aforementioned consideration, the current data provide support for the growing body of evidence suggesting differential responding to pharmacological agents as a function of sex in the Fawn Hooded rat strain [e.g., (11,20)]. Whether these differences are due in part to underlying sex differences in serotonergic efficacy in Fawn Hooded rats is unclear as, to date, there are no studies examining sex differences in serotonergic functioning in these animals. Studies that have examined sex as a variable in responding to LSD have observed no differences between male and female responding to LSD in the Sprague–Dawley or NZBW Hooded strain of rats (12,13). In contrast, sex differences have been observed in the Dark Agouti strain of rat in response to MDMA and these effects have been attributed to an alteration in hepatic enzymes in the female, which result in slower drug clearance (6,7). Whether the current data, suggesting sex differences in the sensitivity to LSD in Fawn Hooded rats, are based on sex differences in CNS serotonergic systems or upon the result of hepatic alterations in pharmacokinetics remains to be determined.

The results of the current study extend the finding that 0.2 mg/kg LSD IP produces a conditioned place preference in male Sprague–Dawley rats (17). Additionally, the inclusion of female animals in the current investigation follows a directive proposed by Bronson et al. (5), who suggested the need for examining sex differences in paradigms involving drugs of abuse. Several studies have indicated that sex/hormonal factors influence the behavioral and toxicological responses to cocaine (4,19,22). Additional work from our laboratory has found sex differences in the lethality observed following administration of the dopamine transport blocker RTI-55 (3). These findings of sex differences in response to drugs of abuse, in conjunction with the documented rise in female drug use (14), strongly suggest a role for continued investigation of sex differences in the examination of the behavioral and toxic effects of psychoactive drugs. Such investigations may help to define potential gender differences in both the etiology and treatment of drug addiction (9,15).

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