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Strain differences in the response to the 5-HT_{1A} receptor agonist, 8-OH-DPAT

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Abstract

Fischer and Sprague–Dawley ovariectomized rats were hormonally primed with estradiol benzoate (EB) and progesterone, and the ability of the 5-HT_{1A} receptor agonist, (±) 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), to inhibit lordosis behavior was examined. Both strains showed rapid inhibition of lordosis behavior following either intraperitoneal or subcutaneous treatment with 8-OH-DPAT. Similarly, in both strains, pretreatment with EB (1 week prior to estrogen and progesterone priming) attenuated the lordosis-inhibiting effects of 8-OH-DPAT. However, Sprague–Dawley females showed a greater decline in lordosis behavior with a lower dose of 8-OH-DPAT than did Fischer females. The strain difference was present in females that had been preprimed with EB as well as in females receiving a single estrogen and progesterone priming. Moreover, strain differences were present across different priming doses of EB. Sprague–Dawley females were also more likely to show flat body posture after injection with 8-OH-DPAT so that the greater sensitivity of this strain to the 5-HT_{1A} receptor agonist was not restricted to the drug's effect on lordosis behavior. These findings lead to the suggestion that, relative to Fischer rats, Sprague–Dawley females are more responsive to the 5-HT_{1A} receptor agonist. Possible explanations for this strain difference are discussed.
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Keywords: Lordosis behavior; Flat body posture; Fischer rats; Sprague–Dawley rats; Females; Sexual behavior; Serotonin syndrome

1. Introduction

The female rat lordosis reflex (arching of the back, elevation of the rump, dorsoflexion of the tail, and extension of the neck) in response to the male's mount is dependent on the gonadal hormone, estrogen, and is facilitated by the gonadal hormone, progesterone (Pfaff and Modianos, 1985). However, multiple neurotransmitters, including serotonin (5-HT), can modulate the behavior (Aiello-Zaldivar et al., 1992; Long et al., 2000; Meyerson, 1964; Uphouse, 2001). 5-HT's control of lordosis is complex and may include 5-HT's activation of several 5-HT receptors (Long et al., 2000; Uphouse, 2001). However, the 5-HT_{1A} receptor appears to account for a major portion of the inhibition of lordosis behavior that follows CNS increases in activity of the 5-HT system (Long et al., 2000; Uphouse, 2001).

In prior studies, wez examined the potency of the 5-HT_{1A} receptor agonist, (±) 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT), in inhibiting lordosis behavior in ovariectomized rats that had been hormonally primed with estradiol benzoate (EB) and progesterone. When rats received a single sequence of priming, a ventromedial hypothalamic (VMH) infusion as low as 100 ng 8-OH-DPAT or a systemic injection as low as 0.15 mg/kg 8-OH-DPAT reduced lordosis behavior in most animals tested (Jackson and Uphouse, 1996, 1998; Trevino et al., 1999; Uphouse et al., 1994). However, when rats were tested after prepriming with EB 1 week earlier, there was a four- to fivefold reduction in the systemic potency of the drug and at least a 10-fold reduction in the potency of drug infusion into the VMH (Jackson and Uphouse, 1996; Trevino et al., 1999).

Our initial observation that prepriming with EB attenuated the later effect of 8-OH-DPAT on female lordosis behavior was not anticipated. Since ovariectomized rats can vary in their response to EB treatment, it is not uncommon for experimenters to hormonally prime and prescreen rats for sexual receptivity 1–2 weeks prior to use in neuropharmacological studies. In other cases, ovariectomized females have been subjected to multiple sequen-

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ces of hormonal primings so that several drug manipulations could be examined within each female. The use of such repeated primings rests upon the assumption that prior hormonal priming does not interact with drug treatment. In the case of the 5-HT_{1A} receptor modulation of lordosis behavior, this assumption proved to be incorrect.

However, our experiments were performed with Fischer females while Sprague–Dawley females have been used in many other laboratories. Therefore, we considered the possibility that the Fischer strain was unique in the response to prepriming with EB. Fischer rats have been reported to be especially sensitive to the development of pituitary tumors after treatment with diethylstibesterol (Wiklund and Gorski, 1982; Ying et al., 1996) and may show a higher sensitivity to estrogen-like xenobiotics (Mendelson, 1992). Therefore, it was possible that the Fischer strain was unusually sensitive to the hormone treatment and that hormonal modulation of the behavioral response to the 5-HT_{1A} receptor agonist did not occur in other rat strains.

In the following studies, ovariectomized Fischer and Sprague–Dawley rats were hormonally primed with EB and progesterone and the lordosis-inhibiting effects of 8-OH-DPAT were examined. Sprague–Dawley rats were selected for this strain comparison because they are reported to differ from Fischer rats on a variety of behavioral and neurochemical parameters (Burnet et al., 1994; Rosecrans et al., 1986; Yoon et al., 1999) and because Sprague–Dawley rats have been widely employed in neuroendocrinological and neuropharmacological investigations. Both strains showed a decrease in lordosis behavior after treatment with 8-OH-DPAT. Prepriming with EB attenuated the decline in both strains. However, with or without hormonal prepriming, Sprague–Dawley rats appeared to be more sensitive than Fischer rats to the lordosis inhibiting effects of the 5-HT_{1A} receptor agonist.

2. Materials and methods

2.1. Materials

Fischer (F344) and Sprague–Dawley female rats were purchased from Sasco Laboratories (Wilmington, MA) at 6–8 weeks of age. 8-OH-DPAT was purchased from Research Biochemicals International (Natick, MA). Suture material was obtained from Butler (Arlington, TX) and methoxyflurane (Metofane) was purchased from Pitman Moore (Mundelein, IL). EB and progesterone were obtained from Sigma (St. Louis, MO). All other supplies came from Fisher Scientific (Houston, TX).

2.2. General methods

2.2.1. Surgery, hormone, and 8-OH-DPAT treatments

Fischer and Sprague–Dawley females were age-matched for the experiments (approximately 12–14 weeks of age at the time of behavioral testing). As a result, the body weights of the two strains differed. Fischer females weighed approximately 150 g and Sprague–Dawley females weighed approximately 250 g when they were used in the experiments. Rats were housed two or three per cage in polycarbonate shoebox cages in a colony room with a 12:12 light–dark cycle (lights on at 12 midnight) with ad lib. access to food and water. Approximately 2 weeks after their arrival at the Texas Woman's University, rats were anesthetized with Metofane and ovariectomized. For a single hormonal priming, 2 weeks after ovariectomy, rats were injected with EB followed 48 h later with progesterone. For prepriming conditions, rats were injected with EB and progesterone 7 days after prior treatment with either EB (Experiment 1) or EB and progesterone (Experiment 2). The effects of 8-OH-DPAT were examined 4–6 h after progesterone treatment. In Experiment 4, an additional group of rats (given two injections of sesame seed oil in a single priming sequence) were included in the study. Hormones were dissolved in sesame seed oil and were administered subcutaneously. EB was administered in a volume of 0.1 ml/150 g body weight. Progesterone was given in a volume of 0.1 ml/rat. 8-OH-DPAT was dissolved in saline and was administered in a volume of 0.1 ml/100 g body weight. In the first experiment, injections were given intraperitoneally to replicate those conditions used in our earlier experiments with Fischer rats. In later experiments, injections were given subcutaneously since this is the route of injection employed by most investigators who have examined effects of 8-OH-DPAT on the serotonin behavioral syndrome.

2.2.2. Behavioral testing—sexual receptivity

Prior to injection with 8-OH-DPAT, the female was placed in the home cage of a sexually experienced male and her sexual behavior was recorded for a minimum of 10 mounts (pretest). The female was then removed, injected with 8-OH-DPAT, and returned to the male's cage. Sexual behavior was recorded for the next 30 min and results were divided into 5-min intervals. Sexual receptivity was quantified by the lordosis-to-mount (L/M) ratio (e.g., number of lordosis responses by the female divided by the number of mounts by the male) as previously described (Jackson and Uphouse, 1996; Uphouse et al., 1992). The quality (e.g., degree of arching of the back) of each lordosis response was scored as previously described (Uphouse et al., 1992). The lordosis behavior of an individual female was considered to be inhibited if the L/M ratio was less than 0.7 for two consecutive 5-min intervals. Lordosis quality was considered to be reduced if the quality of the reflex was reduced 0.5 units for two consecutive testing intervals. These criteria were based on observations that sexually receptive, ovariectomized, hormone-primed females and proestrous females maintain L/M ratios and lordosis quality above these levels for the duration of the testing period (Jackson and Uphouse, 1996, 1998; Uphouse et al., 1992, 1994).

Each female was categorized as either resistive or nonresistive toward the male's attempts to mount. Resistive females boxed, ran from, and sometimes even attacked the male.

2.2.3. Behavioral testing—flat body posture

Increases in CNS 5-HT result in a characteristic set of behaviors known as the "serotonin behavioral syndrome" (Jacobs, 1976; Tricklebank, 1985). Of these behaviors, flat body posture and forepaw treading are elicited by activation of brainstem postsynaptic 5-HT_{1A} receptors (Jacobs and Klemfuss, 1975; Middlemiss et al., 1985; Tricklebank et al., 1985). Flat body posture was recorded when the rat showed an outstretched posture with the abdomen resting close to the cage floor. Severity of flat body posture was scored with a modification to that described by Tricklebank et al. (1985). Since the doses of 8-OH-DPAT in the present experiment were considerably lower than those usually reported to elicit components of the 5-HT syndrome (Larsson et al., 1990; Smith and Peroutka, 1986; Tricklebank et al., 1985), the rating scale was modified to allow for ranking of the comparatively low severity scores anticipated to result from low doses of 8-OH-DPAT. Severity scores were recorded from 0 to 3 as follows: 0=absent; 0.5=equivocal, female had flat body posture but was able to respond to external stimulus (e.g., male mount) by locomotion without flat posture; 1=mild severity; 2=moderate severity with female showing little attempt to move around the cage; 3=high severity with female clearly unable to direct movements. Forepaw treading was recorded when the rat showed repetitive movements of the forepaws. Headweaving (weaving of the head from side to side), straub tail, and gnawing (repetitive gnawing on a food pellet placed in the observation cage), also reported to occur following treatment with 8-OH-DPAT, were also recorded. At the conclusion of the behavioral observation, the number of fecal boli in the cage was recorded.

2.2.4. Statistical procedures

Data for L/M ratios were evaluated by repeated measures ANOVA with time after 8-OH-DPAT as the repeated factor. Differences between treatment (number and/or hormone and/or dose of drug), within time after 8-OH-DPAT, were evaluated by the Tukey's test. Time-dependent effects (within treatment) were evaluated by Dunnett's test. Data for components of the 5-HT behavioral syndrome (Experiment 4) were analyzed by three-factor ANOVA with strain, dose, and hormonal treatment as main effects. The statistical reference was Zar (1996) and an alpha level of .05 was required for rejection of the null hypothesis.

2.3. Specific experiments

2.3.1. Experiment 1

Twenty-eight Fischer and 29 Sprague–Dawley ovariectomized females were used. Rats were injected with EB (0.10 or 0.17 μg/g body weight) followed 48 h later with 500 μg progesterone. For prepriming 7 days earlier, rats received the same dose of EB but did not receive progesterone. Four to six hours after progesterone, rats were tested for sexual receptivity before and after intraperitoneal injection with 0.15 mg/kg 8-OH-DPAT. An individual rat was used only once for either priming (EP condition) or prepriming (EEP condition). Testing of rats was counterbalanced so that EP and EEP rats of each strain were tested on the same day.

2.3.2. Experiment 2

In the second experiment, 29 Fischer and 32 Sprague–Dawley ovariectomized females were hormonally primed with 0.17 μg/g EB 2 weeks after ovariectomy. Progesterone (500 μg, in sesame seed oil) was injected 48 h later. Four to six hours after progesterone, rats were pretested for sexual receptivity as described above. After the behavioral pretest, rats were injected subcutaneously with 0.0125, 0.025, 0.05, 0.075, or 0.1 mg/kg 8-OH-DPAT. Behavioral testing continued for 30 min after injection and sexual receptivity was recorded as described above. The presence or absence of flat body posture was scored for each rat during each 5-min interval after injection with 8-OH-DPAT.

One week after this first behavioral testing, females were again hormonally primed with 0.17 μg/g EB followed 48 h later by 500 μg progesterone. Sexual receptivity and flat body posture were scored as for the first week. Each rat received the same dose of 8-OH-DPAT she had received the prior week. Due to experimenter error, the five Sprague–Dawley and one Fischer females were not tested in the second week.

2.3.3. Experiment 3

In the third experiment, two lower doses of EB were used in a single hormonal priming regimen, initiated 2 weeks after ovariectomy. Fischer and Sprague–Dawley ovariectomized females were primed with 0.068 or 0.017 μg/g EB followed 48 h later with 500 μg progesterone. Four to six hours after progesterone, rats were pretested for sexual receptivity and then injected subcutaneously with 0.025 mg/kg 8-OH-DPAT. The female was returned to the male's cage and behavior was monitored as described above.

2.3.4. Experiment 4

In the final experiment, ovariectomized Fischer and Sprague–Dawley females were injected with 0.17 μg/g EB followed 48 h later with 500 μg progesterone. A second group of each strain was injected with sesame seed oil at each of the injection times. Four to six hours after the second injection, rats were injected subcutaneously with 0.075, 0.10, or 0.25 mg/kg 8-OH-DPAT and components of the 5-HT behavioral syndrome were monitored for 30 consecutive minutes after injection. Data were recorded as the number of minutes that each behavior was present and for the maximum severity score for flat body posture.

3. Results

3.1. Experiment 1: Strain differences after intraperitoneal treatment with 8-OH-DPAT

The effects of 0.15 mg/kg ip 8-OH-DPAT on lordosis behavior of Fischer and Sprague–Dawley females are shown in Fig. 1. There was a significant main effect of strain [$F(1,49) = 11.39$, $P \leq .002$] and number of EB primings [$F(1,49) = 16.61$, $P \leq .0002$], as well as a significant interaction among strain, dose of EB, and number of EB treatments [$F(1,49) = 5.68$, $P \leq .021$]. The main effect of dose of EB was not significant [$F(1,49) = 1.14$, $P > .05$], but there was a significant interaction among the repeated factor, time after injection with 8-OH-DPAT, dose of EB priming, and the number of EB treatments [$F(6,294) = 2.13$, $P \leq .05$]. This interaction resulted from the nearly total attenuation of the lordosis-inhibiting effects of 0.15 mg/kg 8-OH-DPAT in Fischer rats that had been preprimed with 0.17 μg/g EB (EEP rats). Fischer rats preprimed with 0.17 μg/g EB and then primed with 0.17 μg/g EB and progesterone (EEP rats) prior to injection with 8-OH-DPAT had significantly higher L/M ratios than Fischer rats given a single hormonal priming (EP rats). This difference was present at every time interval after injection with 8-OH-DPAT [Tukey's, all $q(294,8) \geq 4.29$, $P \leq .05$]. EEP Sprague–Dawley rats did not differ from EP Sprague–Dawley rats.

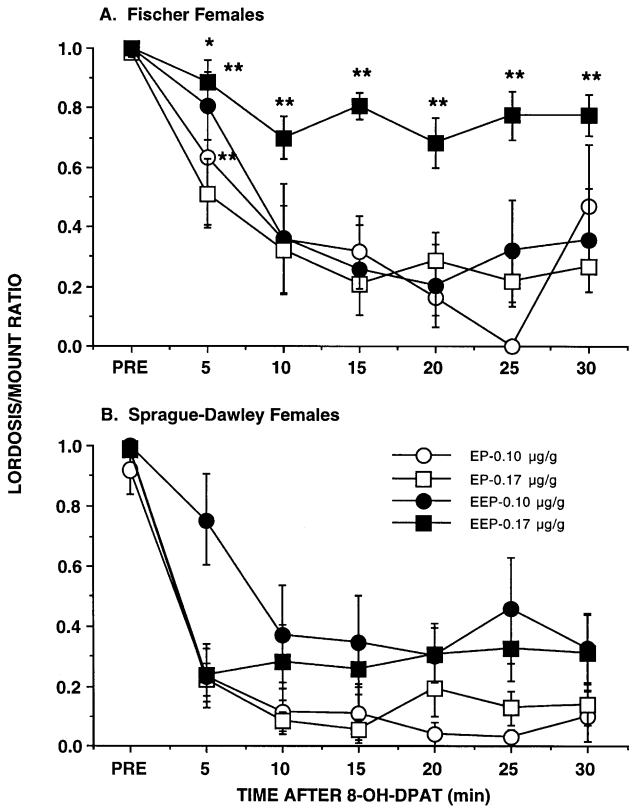

Fig. 1. Strain differences in response to intraperitoneal treatment with 0.15 mg/kg 8-OH-DPAT. Fischer and Sprague–Dawley ovariectomized rats were hormonally primed with either 0.10 or 0.17 μg/g body weight EB followed 48 h later with 500 μg progesterone. Rats of each strain were separated into two groups: EP rats received a single hormonal priming; EEP rats received hormonal priming 7 days after pretreatment with EB. The *n*'s for Fischer rats given 0.10 or 0.17 μg/g were 5 and 7, respectively, for the EP condition and 6 and 10, respectively, for the EEP condition. The *n*'s for Sprague–Dawley rats for the two doses of EB were 5 and 7, respectively, for the EP condition and 7 and 10, respectively, for the EEP condition. Four to six hours after progesterone, rats were tested for sexual receptivity and then injected intraperitoneally with 0.15 mg/kg 8-OH-DPAT. Data in the figure are the mean±S.E. L/M ratios before injection with 8-OH-DPAT and for six 5-min intervals after injection. (A) Data for Fischer females. (B) Data for Sprague–Dawley females. The single asterisk in A represents the first interval where there were significant differences between EP and EEP Fischer females given the same treatment with EB. Significant differences were present thereafter. The double asterisks represent intervals where L/M ratios of EEP Fischer rats were significantly higher than L/M ratios of EEP Sprague–Dawley rats.

3.1.1. Strain differences—single EB priming (EP rats)

After a single hormonal priming, there was no significant difference between Sprague–Dawley and Fischer females in the ability of 0.15 mg/kg 8-OH-DPAT to inhibit lordosis behavior. Every rat showed a decline in the L/M ratio by 5 min after injection [Dunnett's, all $q(294,7) \geq 2.57$, $P \leq .05$]. After injection with 8-OH-DPAT, 10/12 Fischer females showed resistance to the male's attempts to mount while 7/12 Sprague–Dawley females exhibited resistive behavior.

The observation of flat body posture was not preplanned in this experiment because, in our earlier studies (Jackson and Uphouse, 1996), we had seldom seen flat body posture in Fischer rats after intraperitoneal treatment with 0.15 mg/kg 8-OH-DPAT. Consistent with our prior observations, only 1/12 Fischer females showed any evidence of flat body posture after a single EB priming. Unexpectedly, 7/12 of the EP Sprague–Dawley females showed flat body posture during the testing interval. The higher occurrence of flat body posture in Sprague–Dawley females probably accounted for the lower resistive behavior in this strain. In the affected Sprague–Dawley females, resistive behavior appeared to increase as the severity of the flat body posture declined. However, after a single hormonal priming, Sprague–Dawley rats with and without flat body posture did not differ significantly in their L/M ratios after injection with 0.15 mg/kg 8-OH-DPAT [$F(1,60) = 0.128$, $P > .05$].

3.1.2. Strain differences—two EB primings (EEP rats)

When rats were primed twice with EB before treatment with progesterone, there was a significant strain difference in the response to 0.15 mg/kg 8-OH-DPAT. After 0.17 μg/g EB priming, 8-OH-DPAT had a significantly smaller effect in Fischer than in Sprague–Dawley females at every interval after 8-OH-DPAT [Tukey's, all $q(294,8) \geq 4.29$, $P \leq .05$]. Fischer and Sprague–Dawley EEP rats preprimed with 0.10 μg/g EB did not differ. These differential effects of strain, dose, and number of EB treatments resulted in significant interactions between each of these effects and

the repeated measure, time after treatment with 8-OH-DPAT [$F(6,294)=3.50$, 3.52, and 3.3, respectively, all $P \leq .003$].

As was true after a single hormonal priming, females injected with 8-OH-DPAT showed resistance to the male's attempts to mount. For the 0.10- and 0.17-μg/g EB priming, respectively, 5/6 and 9/10 Fischer rats were resistive. For Sprague–Dawley, 5/7 and 8/10 females were resistive toward the male. Two of the 16 Fischer females showed slight flat body posture and 5/19 Sprague–Dawley females showed flat body posture during the 30-min test period after injection with 8-OH-DPAT. In contrast to the findings after a single hormonal priming, after two EB primings, Sprague–Dawley rats that showed flat body posture had significantly lower L/M ratios than did Sprague–Dawley rats that did not show flat body posture [$F(1,15)=8.52$, $P \leq .02$]. Nevertheless, even when females with flat body posture were excluded from the ANOVA, there was still a significant interaction between dose of EB and strain [$F(1,22)=7.00$, $P \leq .02$]. When rats with flat body posture were excluded, Sprague–Dawley females that had been preprimed with EB showed a significantly smaller decline in the L/M ratio after 8-OH-DPAT than did rats given a single EB priming [$F(1,16)=7.71$, $P \leq .02$].

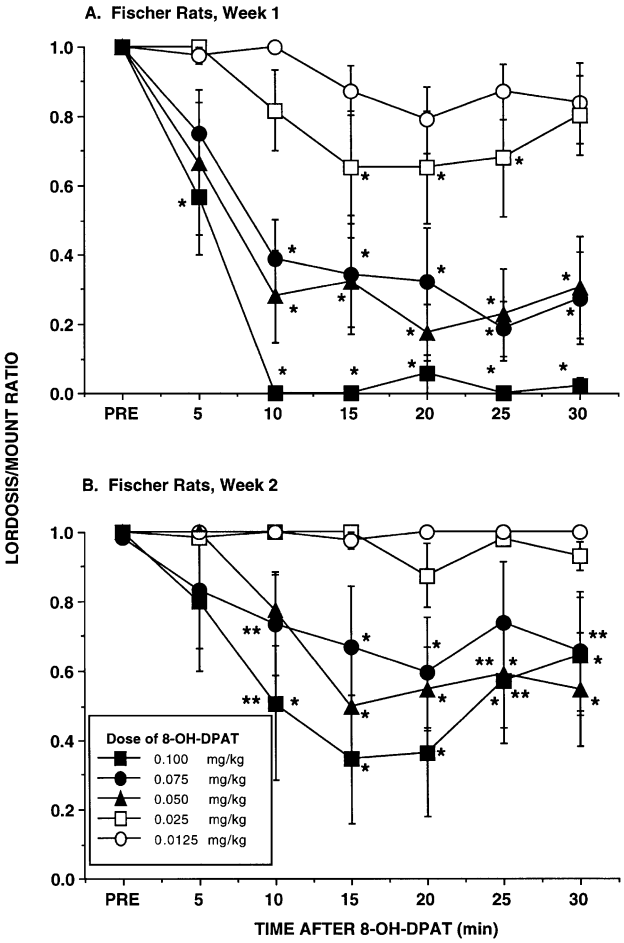

Fig. 2. Dose-dependent effects of subcutaneous 8-OH-DPAT in Fischer females. Fischer ovariectomized rats were hormonally primed with 0.17 μg/g body weight EB followed 48 h later with 500 μg progesterone. Four to six hours after progesterone, rats were tested for sexual behavior and then injected subcutaneously with 0.0125, 0.025, 0.05, 0.075, or 0.10 mg/kg 8-OH-DPAT. The *n*'s were 5, 6, 6, 7, and 5, respectively. One week later, rats received an identical treatment. The *n*'s for Week 2 were 5, 6, 6, 6, and 5, respectively. Data in the figure are the mean±S.E. L/M ratios before injection with 8-OH-DPAT and for six 5-min intervals after injection. (A) Data for the first week of the experiment. (B) Data for Week 2. Single asterisks indicate intervals where the L/M ratio was significantly different from the pretest interval. Double asterisks indicate significant differences between Weeks 1 and 2.

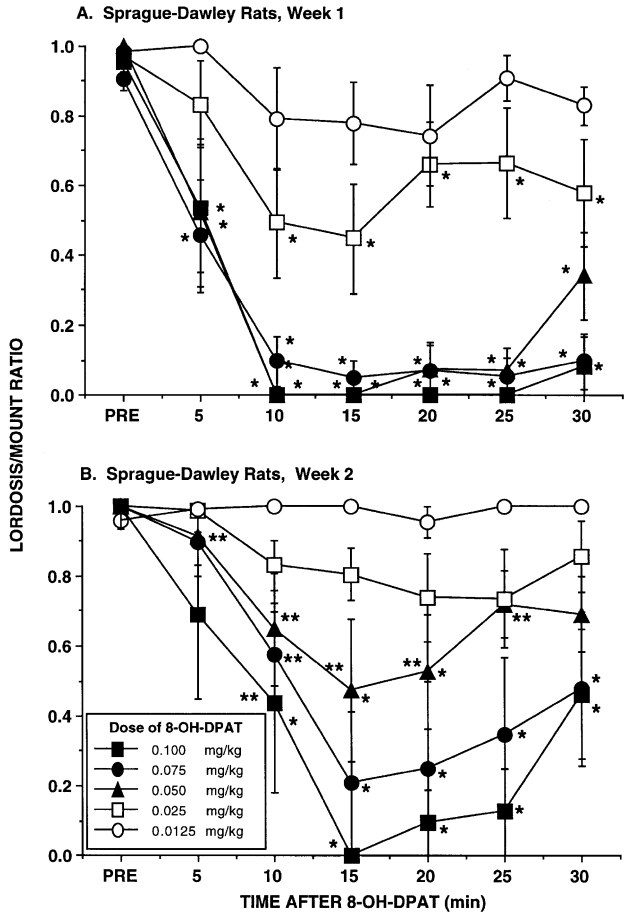

Fig. 3. Dose-dependent effects of subcutaneous 8-OH-DPAT in Sprague–Dawley females. Sprague–Dawley ovariectomized rats were hormonally primed with 0.17 μg/g body weight EB followed 48 h later with 500 μg progesterone. Four to six hours after progesterone, rats were tested for sexual behavior and then injected subcutaneously with 0.0125, 0.025, 0.05, 0.075, or 0.1 mg/kg 8-OH-DPAT. The *n*'s were 5, 7, 7, 7, and 6, respectively. One week later, rats received an identical treatment. The *n*'s for Week 2 were 5, 7, 6, 5, and 4, respectively. Data in the figure are the mean±S.E. L/M ratios before injection with 8-OH-DPAT and for six 5-min intervals after injection. (A) Data for the first week of the experiment. (B) Data for Week 2. Single asterisks indicate intervals where the L/M ratio was significantly different from the pretest interval. Double asterisks indicate significant differences, within dose of 8-OH-DPAT, between Weeks 1 and 2.

3.1.3. Strain differences—other factors

The presence of flat body posture may have affected the male's interest in the female because there were slight strain differences in the average number of mounts per test interval [$F(1,52)=4.18$, $P\leq.05$; mean ± S.E. for Fischer and Sprague–Dawley were 7.5 ± 0.49 and 5.9 ± 2.7, respectively] and these differences were eliminated (mean ± S.E. were 7.42 ± 0.53 and 6.42 ± 0.35, respectively) when rats with flat body posture were omitted from the comparison ($P>.05$). In previous reports, we have noted that lordosis quality, when ordosis occurs, is less affected by 8-OH-DPAT than is the L/M ratio. In the present experiment, most EP rats had L/M ratios of 0 during several postinjection intervals, so statistical comparison of lordosis quality across time after injection was not possible. However, there were no obvious strain differences in the lordosis quality.

3.2. Experiment 2: Strain differences after subcutaneous treatment with 8-OH-DPAT

In the second experiment, each female was injected subcutaneously with one of several doses of 8-OH-DPAT on the first and second week of EB and progesterone priming. Although the dose of 8-OH-DPAT varied between females, each female received the same dose of 8-OH-DPAT each week. Similar to the effects following intraperitoneal treatment with 8-OH-DPAT, there was a significant difference between Sprague–Dawley and Fischer females [ANOVA for main effect of strain, $F(1,91)=9.34$, $P\leq.003$] (Figs. 2 and 3 and Table 1).

For both strains there was a significant reduction in the L/M ratio after injection with 8-OH-DPAT [$F(6,546)=93.75$, $P\leq.0001$]. The decline in the L/M ratio was dose-dependent [ANOVA for dose of 8-OH-DPAT, $F(4,91)=35.24$, $P\leq.0001$]. For both Fischer and Sprague–Dawley females, 8-OH-DPAT was less effective in reducing lordosis behavior on the second relative to the first week of the experiment but, on each week of the study, the 5-HT_{1A} receptor agonist appeared to be less potent in Fischer than in Sprague–Dawley females. Thus, there were significant interactions between the repeated factor (time after injection with 8-OH-DPAT) and each of the main factors [strain, $F(6,546)=2.17$, $p\leq.05$; dose of 8-OH-DPAT, $F(24,546)=7.24$, $P\leq.0001$; and week of treatment, $F(6,546)=8.19$, $P\leq.0001$].

3.2.1. Strain differences—mating behaviors

In the first week of the experiment, in Sprague–Dawley rats, a dose of 8-OH-DPAT as low as 0.025 mg/kg significantly reduced L/M ratios from 10 to 30 min after injection [Dunnett's, all $q(546,8)\geq2.61$, $P\leq.05$]; in Fischer rats given this dose of the drug, a significant decline in the L/M ratio did not occur until 15 min after injection, only lasted from 15 to 25 min after injection, and was of lesser magnitude than in Sprague–Dawley females. For the three highest doses of 8-OH-DPAT (0.05, 0.075, or 0.1 mg/kg), the L/M ratio of both Fischer and Sprague–Dawley females was reduced by 5 min after injection and the reduction persisted for the remainder of the 30-min test period [Dunnett's, all $q(546,8)\geq2.61$, $P\leq.05$]. During the second week of the experiment, the three highest doses of 8-OH-DPAT still reduced L/M ratios in both strains, but the decline was smaller than on the first week; and the onset of inhibition was slower on the second than on the first week of the study. Thus, the dose of 8-OH-DPAT that was required to reduce L/M ratios was lower in

Table 1
Strain differences in response to subcutaneously administered 8-OH-DPAT

Strain	Number of EB+ progesterone	Dose 8-OH-DPAT (mg/kg)	*n*	Reduced L/M ratio	Number of rats with				Maximum severity flat body posture (mean ± S.E.)
					Resistance	Flat body posture	L/M to zero	reduced quality	
Fischer	1	0.100	5	5	2	4	5	1	0.50 ± 0.16
	1	0.075	7	5	7	4	4	6	0.36 ± 0.14
	1	0.050	6	6	6	0	5	6	0
	1	0.025	6	3	5	0	1	2	0
	1	0.0125	5	1	5	0	0	0	0
	2	0.100	5	4	3	4	2	5	0.50 ± 0.158
	2	0.075	6	3	5	2	2	3	0.25 ± 0.17
	2	0.050	6	4	5	0	2	5	0
	2	0.025	6	0	6	0	0	0	0
	2	0.0125	5	0	4	0	0	0	0
Sprague–Dawley	1	0.100	6	6	6	6	6	3	1.16 ± 0.21
	1	0.075	7	7	6	6	6	4	0.79 ± 0.15
	1	0.050	7	7	4	4	6	5	0.29 ± 0.14
	1	0.025	7	5	5	0	1	4	0
	1	0.0125	5	1	2	0	0	0	0
	2	0.100	4	4	0	4	4	3	0.75 ± 0.14
	2	0.075	5	4	2	5	3	2	0.70 ± 0.12
	2	0.050	6	5	3	3	3	4	0.21 ± 0.10
	2	0.025	7	3	3	0	0	1	0
	2	0.0125	5	0	1	0	0	1	0

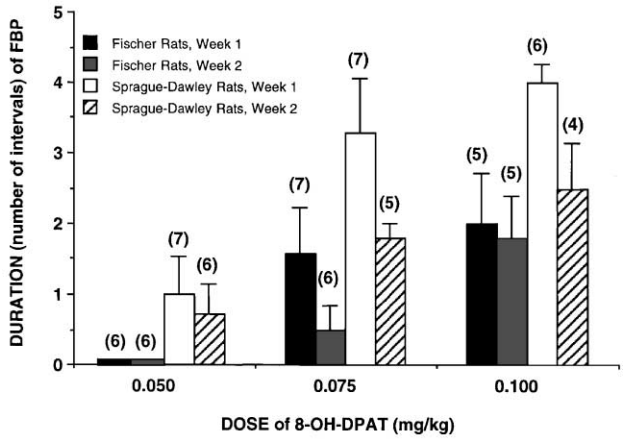

Fig. 4. Strain differences in flat body posture after 8-OH-DPAT. Flat body posture was recorded for each rat for each 5-min interval during the 30-min period after injection with 8-OH-DPAT. Data are the mean±S.E. number of 5-min intervals during which flat body posture was present for Fischer and Sprague–Dawley females after the three highest doses of 8-OH-DPAT. Numbers in parentheses refer to the *n*'s in each group.

Sprague–Dawley than in Fischer rats, and this was true for each week of the experiment. However, for both strains, a higher dose of 8-OH-DPAT was required in Week 2 relative to Week 1.

For several doses of 8-OH-DPAT, females from both strains had L/M ratios of zero after injection with the 5-HT_{1A} receptor agonist (Table 1). Thus, it was not possible to statistically compare lordosis quality across the strains and doses of the drug.

As in the first experiment, Sprague–Dawley females received fewer overall mounts than did Fischer rats [$F(1,98)=12.21$, $P\leq.0007$; mean±S.E. mounts per test interval for Sprague–Dawley and Fischer were 6.41±0.20 and 7.74±0.21, respectively], but there was no significant interaction between strain and any other factor (all $P>.05$). Strain differences in mounts were eliminated when rats without flat body posture were removed from the comparison [$F(1,79)=0.497$, $P>.05$; mean±S.E. mounts per test interval for Sprague–Dawley and Fischer were 7.45±0.27 and 7.77±0.24, respectively].

3.2.2. Strain differences—flat body posture

In the second experiment, assessment of flat body posture was preplanned, as described in the Methods. The numbers of Sprague–Dawley and Fischer females that showed flat body posture during the test period are shown in Table 1. Flat body posture was not observed for either strain at the two lowest doses of 8-OH-DPAT. However, for the three highest doses of the drug, Sprague–Dawley females were more likely to show flat body posture than were Fischer females. Sprague–Dawley females showed a longer duration of flat body posture (Fig. 4) and a greater maximum severity flat body posture score (Table 1) than did Fischer rats [for duration and maximum severity, $F(1,97)=16.83$ and 21.50, respectively, $P\leq.002$]; and strain interacted significantly with the dose of 8-OH-DPAT [$F(4,97)=3.15$ and 4.05, respectively, $P\leq.02$]. Week of testing did not affect the maximum severity score, but the duration of flat body posture decreased on the second, relative to the first week of treatment [$F(1,97)=6.29$, $P\leq.02$]. No other interactions with week of testing were significant.

The strain difference in flat body posture probably contributed to the strain difference in effects of 8-OH-DPAT on lordosis behavior because there was a significant negative correlation ($r=-.591$, $P\leq.0001$) between the average L/M ratio and the maximum flat body posture score. However, at the three highest doses of the drug, there were too few Sprague–Dawley females without flat body posture to compare animals with and without flat body posture.

3.3. Experiment 3: Strain comparisons with single, lower-dose EB priming

In the third experiment, ovariectomized Sprague–Dawley and Fischer rats were hormonally primed once only with either 0.068 or 0.017 μg/g EB followed 48 h later with 500 μg progesterone. All rats were injected with 0.025 mg/kg sc 8-OH-DPAT, a dose of the 5-HT_{1A} receptor agonist that produced only a small decline in the L/M ratio of either strain following priming with 0.17 μg/g EB. Even with these lower doses of EB priming, the lordosis-inhibiting effects of 8-OH-

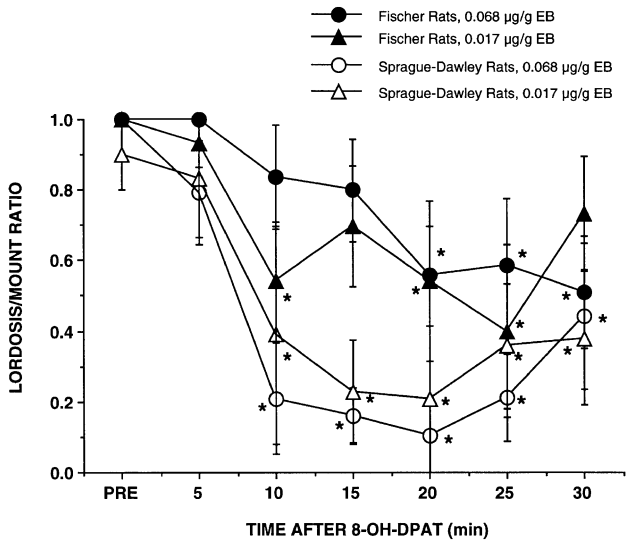

Fig. 5. Effects of 0.025 mg/kg 8-OH-DPAT after relatively low doses of EB priming. Fischer and Sprague–Dawley ovariectomized rats were hormonally primed with 0.017 or 0.068 μg/g EB followed 48 h later with 500 μg progesterone. The *n*'s for Sprague–Dawley rats for the 0.068 and 0.017 μg/g doses of EB were 4 and 4, respectively; for Fischer rats, the *n*'s, were 5 and 5, respectively. Data in the figure are the mean±S.E. L/M ratios before injection with 8-OH-DPAT and for six 5-min intervals after injection. Asterisks indicate intervals where the L/M ratio was significantly reduced relative to the pretest.

DPAT were greater in Sprague–Dawley than in Fischer females [ANOVA for strain, $F(1,13)=7.39$, $P\leq.02$] (Fig. 5).

3.4. Experiment 4: Strain differences in 5-HT syndrome and other behaviors

Strain differences in the effects of 8-OH-DPAT on flat body posture, forepaw treading, gnawing, and number of feces are shown in Figs. 6 and 7. Straub tail and head-weaving were too infrequently observed to consider any strain comparison meaningful. For flat body posture, strain [$F(1,49)=4.3$, $P\leq.05$] and dose of 8-OH-DPAT [$F(2,49)=8.9$, $P\leq.0005$] effects were evident for the duration and maximum severity [$F(1,49)=4.04$, $P\leq.05$, and $F(2,49)=4.27$, $P\leq.02$, respectively] with Sprague–Dawley showing higher scores than Fischer rats (Fig. 6). For neither measure was hormone a significant factor and no interaction terms were significant (all $P>.05$).

Dose of 8-OH-DPAT significantly influenced the occurrence of forepaw treading [$F(2,41)=9.77$, $P\leq.0003$], but there were no significant effects of strain, hormone, or any interaction term (all $P>.05$). In contrast, strain (but not dose) differences were present for gnawing and for fecal boli. Fischer rats engaged in more gnawing on the food pellets [ANOVA, $F(1,47)=23.7$, $P<.0001$] and had more fecal boli [$F(1,37)=17.81$, $P\leq.0002$] than did Sprague–Dawley females (Fig. 7). No other factors were significant.

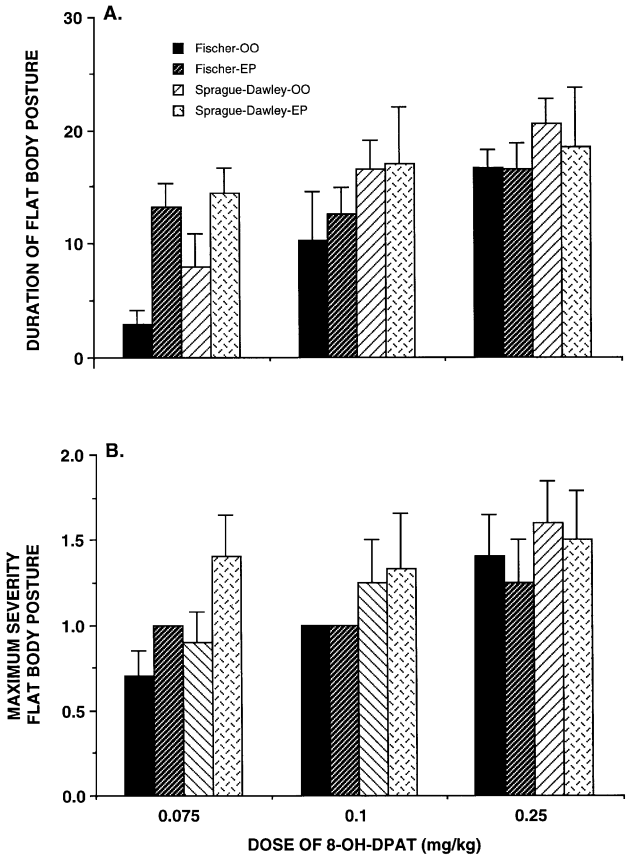

Fig. 6. Strain differences in flat body posture after 8-OH-DPAT. Fischer and Sprague–Dawley ovariectomized rats were injected with oil or 0.17 μg/g EB and 500 μg progesterone as described in the Methods. Four to six hours after the second injection, rats were injected subcutaneously with 0.075, 0.10, or 0.25 mg/kg 8-OH-DPAT. Rats were observed for 30 consecutive min for the presence of flat body posture. A Mean±S.E. duration of flat body posture in minutes. B Mean±S.E. maximum severity scores.

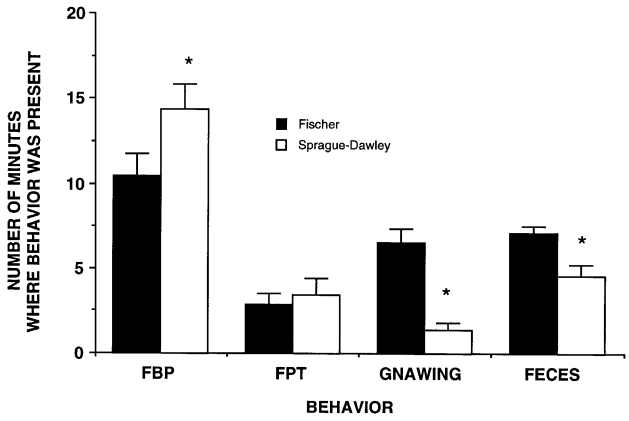

Fig. 7. Strain differences in forepaw treading, gnawing, and number of fecal boli. Fischer and Sprague–Dawley ovariectomized rats were injected with oil or 0.17 μg/g EB and 500 μg progesterone as described in the Methods. Four to six hours after the second injection, rats were injected subcutaneously with 0.075, 0.10, or 0.25 mg/kg 8-OH-DPAT. Rats were observed for 30 consecutive minutes for the presence of flat body posture, forepaw treading, and gnawing. Fecal boli in the cage at the end of the 30-min period were recorded. Data are the mean±S.E. number of minutes of flat body posture, forepaw treading, and gnawing, and total number of fecal boli. Data have been collapsed across hormone and dose of 8-OH-DPAT for presentation. Asterisks indicate significant differences between strains.

4. Discussion

Prior treatment with EB reduced the lordosis-inhibiting effects of 8-OH-DPAT in both Fischer and Sprague–Dawley rats. However, relative to Fischer females, Sprague–Dawley females were more sensitive to the effects of 8-OH-DPAT. This increased sensitivity was evident both in 8-OH-DPAT's reduction of lordosis behavior and in the occurrence of flat body posture, and the greater sensitivity was evident after either one or two primings with EB. Since, lordosis behavior can only be monitored in rats after hormonal priming, we cannot be sure that the greater lordosis-inhibiting potency of 8-OH-DPAT in Sprague–Dawley females is independent of strain differences in response to gonadal hormones. However, strain differences were present across a wide range of doses of EB, including the relatively high dose of 0.17 μg/g EB. Moreover, Sprague–Dawley females showed more flat body posture than did Fischer females, and this strain difference was equally evident in ovariectomized rats that were injected with sesame seed oil and in those that received hormonal priming.

The mechanisms responsible for these strain differences have not yet been determined. 5-HT_{1A} receptors in brain-

stem locations are believed to be responsible for the effects of 8-OH-DPAT on flat body posture (Jacobs and Klemfuss, 1975) while 5-HT_{1A} receptors in the VMH can account for the lordosis-inhibiting effects of the drug (Uphouse et al., 1992). Thus, whatever mechanisms are responsible for the lower sensitivity of Fischer females to 8-OH-DPAT, they are not restricted to hypothalamic sites. However, both flat body posture and inhibition of lordosis behavior after 8-OH-DPAT are thought to be mediated by activation of 5-HT_{1A} receptors that are postsynaptic to 5-HT neurons (Tricklebank et al., 1985). Thus, the current studies allow the suggestion that Fischer and Sprague–Dawley may differ in their postsynaptic response to the 5-HT_{1A} receptor agonist.

It is also possible that Fischer rats have less extracellular 5-HT so that higher concentrations of the receptor agonist would be required to elicit the 5-HT_{1A} receptor-mediated behaviors. Burnet et al. (1994, 1996) found a greater abundance of 5-HT transporter mRNA in the raphe of Fischer relative to Sprague–Dawley rats. If Fischer rats have a more efficient reuptake of released 5-HT, then a greater concentration of the 5-HT_{1A} receptor agonist might have been required to activate postsynaptic 5-HT_{1A} sites. However, Fischer rats have generally been claimed to have a hyperfunctional, rather than a hypofunctional, 5-HT system (Jackson and Uphouse, 1996; Rosecrans et al., 1986). Higher tissue levels of 5-HT and its metabolite, 5-hydroxyindole-acetic acid (5-HIAA), have been reported in hippocampus of Fischer relative to Sprague–Dawley females (Burnet et al., 1996; Rosecrans et al., 1986). In contrast, Fischer females had lower 5-HT and 5-HIAA in the hypothalamus than did Sprague–Dawley rats (Burnet et al., 1996) and Rosecrans et al. (1986) reported that Fischer rats had a lower 5-HIAA/5-HT ratio in hypothalamus than did Sprague–Dawley rats. Thus, brain regions may vary in how the two strains differ in synaptic availability of 5-HT. Finally, since 8-OH-DPAT was administered systemically, we cannot rule out the possibility that a differential effect of 8-OH-DPAT on presynaptic somatodendritic autoreceptors of the dorsal raphe nucleus contributed to the strain difference.

In summary, while both Fischer and Sprague–Dawley females showed an attenuated response to 8-OH-DPAT after prepriming with gonadal hormones, Sprague–Dawley females showed a greater sensitivity to the 5-HT_{1A} receptor agonist. Fischer and Sprague–Dawley rats are used by multiple research laboratories and findings obtained in one strain are often generalized to the other strain. It is, therefore, important to note that significant dose–response differences may exist between these two strains.

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