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# Gender and Estrous Cycle Effects of the 5-$HT_{1A}$ Agonist, 8-OH-DPAT, on Hypothalamic Serotonin

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MASWOOD, S., G. STEWART AND L. UPHOUSE. *Gender and estrous cycle effects of the 5-$HT_{1A}$ agonist, 8-OH-DPAT, on hypothalamic serotonin.* PHARMACOL BIOCHEM BEHAV **51**(4) 807–813, 1995.—Effects of the 5-$HT_{1A}$ agonist, 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT; 0.04, 0.25, or 1.0 mg/kg), on hypothalamic serotonin (5-HT), 5-hydroxyindoleacetic acid (5-HIAA), and their ratio were determined in adult male rats and in diestrous, proestrous, and estrous female rats. Consistent with its action at the somatodendritic 5-$HT_{1A}$ autoreceptor, 8-OH-DPAT decreased the 5-HIAA/5-HT ratio, but the decrease was least evident in proestrous females and in males. Similar to hypothalamic tissue, there was also a decline in the 5-HIAA/5-HT ratio in the hippocampus after treatment with 0.25 mg/kg 8-OH-DPAT. When ovariectomized rats were treated with oil or estradiol benzoate followed 48 h later by oil or progesterone, 0.25 mg/kg 8-OH-DPAT produced a decrease in the hypothalamic 5-HIAA/5-HT ratio in every group except those rats treated with progesterone without estrogen priming. Treatment with estradiol benzoate increased hypothalamic 5-HIAA, and both progesterone and 8-OH-DPAT reduced the metabolite to the level of the ovariectomized control. These results suggest that both estrogen and progesterone contribute to an estrous cycle modulation of the 5-$HT_{1A}$ somatodendritic autoreceptor.

Male/female rats  Proestrus  Estrogen  Progesterone  Serotonin

SEROTONIN (5-HT) has been implicated in the etiology of both depression and anxiety disorders (5,15,17), and male and female rodents differ in several indices of 5-HT function (11,23). Moreover, when both male and female rodents have been compared in either animal behavioral models of anxiety or depression or in the study of antidepressant or antianxiety compounds, the suggestion of sex differences is inescapable (8,10,11,19,23,41). Multiple biochemical indices of 5-HT function vary during the female estrous cycle (3,12,42), and gonadal hormonal effects on 5-HT function are relatively widespread and include apparent effects on synthesis, release, reuptake, and catabolism (3,4,9,27,31,33,44). Most interesting, however, is the report by Lakoski (28) that estrogen reduces the ability of the 5-$HT_{1A}$ receptor agonist, 8-hydroxy-2-(di-*n*-proplyamino)tetralin (8-OH-DPAT), to reduce firing of dorsal raphe (DR) neurons. Somatodendritic 5-$HT_{1A}$ receptors regulate the firing of 5-HT neurons and consequent release of 5-HT from nerve terminals (22,35,37), and these receptors have been emphasized as potential sites involved in the mechanism of action of both typical and atypical (6,24,30,32) antidepressant and antianxiety drugs.

If estrogen reduces the ability of 5-$HT_{1A}$ agonists to decrease DR firing, then estrogen would be expected to increase the release of 5-HT from nerve terminals. Such an increase has been suggested by several investigators (7,9). Progesterone, in contrast, should attenuate the release-enhancing properties of estrogen. GABA and benzodiazepines, which facilitate GABA's action at $GABA_A$ receptors, reduce the firing of 5-HT neurons (14,38), and progesterone metabolites interact with the $GABA_A$ receptor (2,20) and enhance GABAergic stimulation (36).

Although sexual dimorphisms in the response to 5-$HT_{1A}$ agonists have been observed (10,19,41), in few studies have both gender and estrous cycle effects been examined. However, Uphouse et al. (41) reported that 8-OH-DPAT-induced hyperphagia [thought to result from agonist activation of the 5-$HT_{1A}$ autoreceptor (43)] was less prominent in proestrous and estrous females than in diestrous females and in males,

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and the reduced effectiveness of the drug appeared to result from the presence of estrogen (34). Although these findings are consistent with Lakoski's (28) suggestion that estrogen reduced the sensitivity of somatodendritic 5-$HT_{1A}$ autoreceptors, 8-OH-DPAT-induced changes in 5-HT and/or its major metabolite were not examined so it is not possible to rule out potential gender and estrous cycle-dependent differences in postsynaptic 5-HT receptors and consequent differences in eating behavior.

The present series of experiments were designed to examine the effects of 8-OH-DPAT on tissue concentrations of 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA), in male rats and in female rats from the stages of diestrus 2, proestrus, and estrus. The effects of 8-OH-DPAT in hormonally primed, ovariectomized rats were also studied. Theoretically, by decreasing the firing of 5-HT neurons, 8-OH-DPAT should reduce release of 5-HT and thereby reduce the relative amount of 5-HT recovered by the nerve terminal and degraded to 5-HIAA. Therefore, if estrogen and/or progesterone reduce the effectiveness of 8-OH-DPAT on somatodendritic 5-$HT_{1A}$ receptors, then 8-OH-DPAT would be expected to be less effective in proestrous and/or in estrous rats in reducing tissue concentrations of 5-HIAA. In general, this expectation was confirmed by the present studies.

## METHOD

### *Materials*

8-OH-DPAT was purchased from Research Biochemicals Inc. (Natick, MA). All other supplies came from Fisher Scientific (Houston, TX).

### *Treatment of Animals*

Adult male and female rats (CDF-344) were bred in our laboratory from stock obtained from Sasco Laboratories (Omaha, NE). At 25 days of age, rats were weaned and housed with like-sex littermates in polycarbonate shoe-box cages. Animals were housed two or three per cage, in a colony room with a 12L : 12D cycle (lights on at 2400 h) with ad lib access to food (rat chow) and water. When intact female rats were used, they were monitored daily for vaginal cyclicity for at least two complete estrous cycles, as previously described (41). Smears with clusters of nucleated and/or cornified epithelial cells but an absence of leucocytes were judged as proestrous smears; the presence of predominantly cornified cells, a day after a proestrous smear, represented an estrous smear. Leucocytes present alone or together with some nucleated and/or cornified cells indicated a diestrous smear. Proestrous, diestrous 2, or estrous female rats were selected for use in the experiments. Males were matched either by age or by weight with the females and were handled daily prior to use in the experiment. When ovariectomized rats were used, at 60–90 days of age, female rats were ovariectomized bilaterally under methoxyflurane anaesthesia Two weeks later, rats received subcutaneous (SC) (0.1 ml/rat) injections of either sesame oil or estradiol benzoate (25 μg/animal) in sesame oil about 1000 h. Forty-eight hours later, rats were injected SC (0.1 ml/rat) with either sesame oil or progesterone (500 μg/animal).

In the first experiment, proestrous, diestrous 2, and estrous female rats and male rats were injected intraperitoneally (IP) with 0.04, 0.25, or 1.0 mg/kg of 8-OH-DPAT or saline between 1500 and 1700 h and were sacrificed 30 min later. In the second experiment, ovariectomized rats were treated with 0.25 mg/kg of 8-OH-DPAT 4–5 h after the second hormone or oil injection and were sacrificed in pairs (saline or 8-OH-DPAT) with the order of sacrifice counterbalanced across treatments. In the final experiment, intact proestrous, diestrous 2, or estrous rats received 0.25 mg/kg 8-OH-DPAT or saline between 1500 and 1700 h. Injections of 8-OH-DPAT or 0.9% saline were given IP in a volume of 1.0 ml/kg rat and rats were sacrificed by decapitation 30 min later. In all experiments, rats remained in a dark environment during the 30-min interval after injection with 8-OH-DPAT or saline. Red lighting was used to aid experimenter visibility.

All experiments were performed during the dark portion of the light : dark cycle, 3–5 h after lights off, because it is during this time of the day that proestrous rats show high levels of sexual receptivity [a behavior known to be inhibited by 5-$HT_{1A}$ agonists (1,39,40) and facilitated by removal of 5-HT and by lesioning of dorsal raphe neurons (13)]. Furthermore, with this procedure, the hormonal treatment (estrogen + progesterone) was most likely to simulate the proestrous state.

Rats were rapidly decapitated and the hypothalamus (Experiments 1 and 2) or the hypothalamus and hippocampus (Experiment 3) were removed by the procedure of Glowinsky and Iversen (16) and frozen immediately on dry ice. Frozen tissues were weighed to the nearest 0.1 mg and were homogenized by sonication into 10 vol. (for hypothalamus) or 5 vol. (for hippocampus) of 0.05 M perchloric acid containing 0.1% cysteine. Homogenized samples were stored at −80°C until processed for HPLC analysis.

### *HPLC Procedures*

The levels of 5-HT and its principal metabolite, 5-HIAA, were determined by electrochemical detection essentially as described by Kilts et al. (25). The mobile phase (pH adjusted to 3.62) was 0.09 M citric acid, 0.07 M sodium phosphate, 0.10 mM ethylenediamine tetraacetic acid, 2.62 mM sodium octylsulfate, and 8% methanol. The flow rate was 1.0 ml/min and the oxidation potential was 0.75 V. Immediately prior to injection onto the HPLC column, tissue samples were centrifuged at 10,000 rpm for 10 min in a 18.1 rotor in a Beckman J2-21 centrifuge. Samples (20 μl) of the supernatant were used for the determination of 5-HT and 5-HIAA. Samples were analyzed in duplicate. Quantitative determinations were made by comparison with appropriate external standards and data were reported as pmol per mg of original tissue.

With these conditions, quantification was linear at least over the range of 2.5–20 pmol for both 5-HT and 5-HIAA. For recovery estimates of 5-HT and 5-HIAA, tissue was processed in parallel plus or minus the addition of known amounts of the amines. Recovery of 5-HT was 100% and that of 5-HIAA was 86%. No correction for recovery was made in the data analysis.

### *Statistical Methods*

Data for 5-HT, 5-HIAA, and their ratio were analyzed separately. Effects of 8-OH-DPAT in intact animals were evaluated by ANOVA with stage (or sex) and treatment (saline or 8-OH-DPAT) as independent factors. When the effects of gonadal hormones were compared, data were analyzed by matched-pair procedures. When hypothalamic and hippocampal tissue were compared, the data were normalized by computation of the ratio between 8-OH-DPAT-treated rats and the average for the appropriate parameter from the same stage (or sex) saline control. ANOVA was performed on the normalized data. Planned comparisons between individual means were made with Dunnett's or Tukey's tests and Scheffe proce-

dures were used for contrasts. The statistical reference was Zar (46) and an alpha level of 0.05 was required for rejection of the null hypothesis.

## RESULTS

The somatodendritic 5-$HT_{1A}$ autoreceptor agonist, 8-OH-DPAT, dose-dependently decreased the hypothalamic 5-HIAA/5-HT ratio, $F(3, 58) = 10.84$, $p \leq 0.0001$, and the decrease was most evident for 0.25 and 1.0 mg/kg 8-OH-DPAT [Dunnett's $q(58, 4) \geq 4.7$, $p \leq 0.05$] (Fig. 1C). Diestrous and estrous females were significantly different from their appropriate saline control at both 0.25 mg/kg [respectively, Dunnett's $q(58, 4) = 2.85$ and 3.66, $p \leq 0.05$] and 1.0 mg/kg 8-OH-DPAT [respectively, Dunnett's $q(58, 4) = 5.64$ and 3.21, $p \leq 0.05$]. In contrast, males and proestrous females were significantly different from their saline controls only at the highest dose of the drug [Dunnett's $q(58, 4) = 3.48$ and 2.86, $p \leq 0.05$]. Although the magnitude of the decrease appeared to be both gender and estrous cycle dependent, all groups showed some response to 8-OH-DPAT so the overall effect of cycle or gender narrowly escaped statistical significance, $F(3, 58) = 2.42$, $p \leq 0.075$, and there was no treatment × stage interaction ($p > 0.05$).

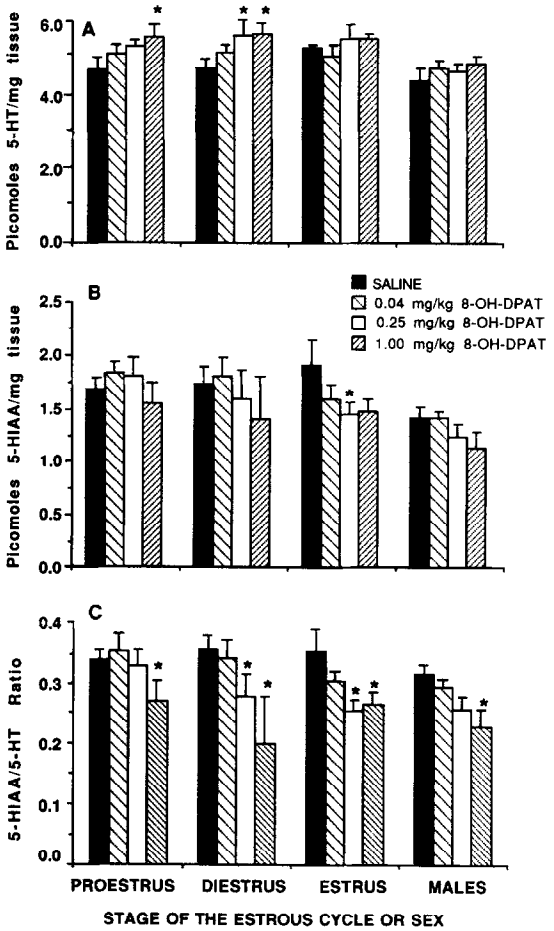


FIG. 1. Effects of different doses of 8-OH-DPAT on hypothalamic 5-HT, 5-HIAA, and the 5-HIAA/5-HT ratio of female and male rats. Proestrous, diestrous, or estrous female or male rats were injected IP with saline or with 0.04, 0.25, or 1.0 mg/kg 8-OH-DPAT. After 30 min, the animals were decapitated; the hypothalamus was dissected and processed as described in the Method section; and the levels of 5-HT, 5-HIAA, and the 5-HIAA/5-HT ratio were determined. The mean ± SE pmol 5-HT and 5-HIAA per mg hypothalamus and their ratio are shown (A), (B), and (C), respectively. The *N*s for saline and the three doses of 8-OH-DPAT were as follows: proestrus 6, 5, 5, 5; diestrus 5, 4, 3, 3; estrus 3, 7, 6, 5; and males 5, 4, 4, 4. *Significant differences from the appropriate saline controls.

An 8-OH-DPAT-induced increase in 5-HT (Fig. 1A) was present in diestrous females at both 0.25 and 1.0 mg/kg doses of the drug [Dunnett's $q(58, 4) = 2.71$ and 2.91, $p \leq 0.05$]; proestrous females were significantly different from their saline control at the highest dose [Dunnett's $q(58, 4) = 3.30$, $p \leq 0.05$]. At no dose of 8-OH-DPAT were estrous females or males significantly different from their saline control (Dunnett's $p > 0.05$). Both stage of the estrous cycle (or sex), $F(3, 58) = 4.39$, $p \leq 0.008$, and drug treatment, $F(3, 58) = 3.48$, $p \leq 0.02$, significantly influenced the hypothalamic level of 5-HT, but there was no overall interaction between treatment and stage of the estrous cycle (or gender) ($p > 0.05$).

There was a dose-dependent decline in hypothalamic levels of 5-HIAA (Fig. 1B) following treatment with 8-OH-DPAT but the overall effect of treatment was only marginally significant, $F(4, 58) = 2.61$, $p \leq 0.059$. A significant effect of stage (or sex), $F(3, 58) = 4.82$, $p \leq 0.004$, resulted from lower levels of 5-HIAA in the males compared to the proestrous or the diestrous females [Tukey $q(58, 4) = 5.06$ and 4.08, $p \leq 0.05$]. The stage by treatment interaction was not significant ($p > 0.05$), but visual inspection of the figure suggested that the level of 5-HIAA was decreased in estrous females at a lower dose of 8-OH-DPAT than in the other groups and that proestrous females were least responsive to 8-OH-DPAT.

The above results suggested that reciprocal modulation of 5-HT and 5-HIAA accounted for the decline in the 5-HIAA/5-HT ratio after 8-OH-DPAT but that changes in 5-HT and 5-HIAA in response to 8-OH-DPAT nonuniformly contributed to the estrous cycle variation in the 8-OH-DPAT-induced decline in the 5-HIAA/5-HT ratio. Because the hypothalamic levels of 5-HT and 5-HIAA were not constant across the estrous cycle, data for 5-HT and 5-HIAA were normalized to their appropriate same-stage saline control so that the response to 8-OH-DPAT could be examined independently of cycle differences in the biogenic amine (Fig. 2). Significant stage effects were present for the 5-HIAA (drug)/5-HIAA (saline) ratio, $F(2, 34) = 4.81$, $p \leq 0.002$, as well as for the 5-HT (drug)/5-HT(saline) ratio, $F(2, 34) = 4.67$, $p \leq 0.02$. As indicated above, proestrous females showed an increase in 5-HT with modest, if any, changes in 5-HIAA; estrous females showed modest changes in 5-HT with more robust reductions in 5-HIAA; and diestrous females showed both an increase in 5-HT and a decline in 5-HIAA. As a result, a decline in the 5-HIAA/5-HT ratio was most evident in the diestrous females.

To pursue the estrous cycle differences in the response to 8-OH-DPAT, we evaluated the effect of 0.25 mg/kg 8-OH-DPAT in ovariectomized rats primed with estrogen and/or progesterone (Fig. 3). Over all hormone treatments, there was no significant effect of 8-OH-DPAT on hypothalamic levels of 5-HT. However, a significant effect of drug treatment, $F(1, 18) = 15.8$, $p \leq 0.009$, as well as a significant hormone treatment by drug treatment interaction, $F(4, 18) = 3.26$, $p \leq 0.05$, was present for 5-HIAA. When 8-OH-DPAT-treated rats were compared to their saline controls, oil/oil-treated females and those treated with estradiol benzoate/oil showed a decline in 5-HIAA [Dunnett's $q(18, 4) = 3.97$ and 5.42, $p \leq 0.05$]. Estradiol benzoate produced an elevation in the level

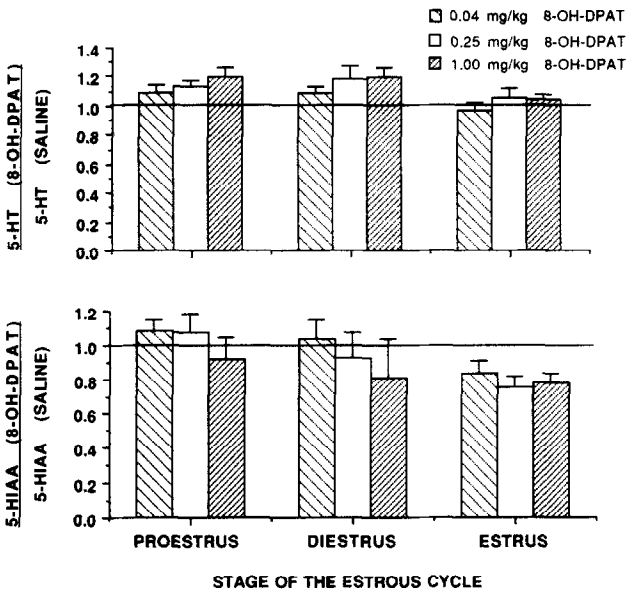


FIG. 2. 5-HT and 5-HIAA after 8-OH-DPAT as a ratio to the saline control. For each 8-OH-DPAT treated female, hypothalamic 5-HT and 5-HIAA (shown in Fig. 2) were divided by the level of the amine and its metabolite in the same-stage saline control. The figure shows the mean ± SE of the resulting ratios.

of hypothalamic 5-HIAA in the saline-treated rats [Dunnett's $q(18, 4) = 2.56$, $p \leq 0.05$], but when estradiol benzoate-primed rats were treated with either progesterone or with 8-OH-DPAT, hypothalamic 5-HIAA was reduced to that of the oil-treated, saline control. Consequently, 8-OH-DPAT failed to further reduce 5-HIAA in animals primed with both estradiol benzoate and progesterone; neither was there a significant decline in 5-HIAA in rats primed with oil and progesterone [respectively, Dunnett's $q(18, 4) = 2.31$ and $0.47$, $p > 0.05$]. However, 8-OH-DPAT decreased the 5-HIAA/5-HT ratio, $F(1, 18) = 42.95$, $p \leq 0.0001$, in all animals except those treated with oil and progesterone [respectively, Dunnett's $q(18, 4) \geq 5.05$, $p \leq 0.05$ and $q(18, 4) \leq 2.56$, $p > 0.05$]. As a result, for the 5-HIAA/5-HT ratio, the drug treatment × hormone treatment interaction was significant, $F(3, 18) = 3.31$, $p \leq 0.05$.

The preceding experiments led to the suggestion that female gonadal hormones (especially progesterone) influenced the effect of 8-OH-DPAT on 5-HT and its metabolite and that the hypothalamic response to the drug differed during the estrous cycle. In the next study, hypothalamic and hippocampal tissues were included to evaluate the specificity of this effect for the hypothalamus (Table 1). Only a single concentration of 8-OH-DPAT (0.25 mg/kg) was examined.

The effects of 8-OH-DPAT on hypothalamic 5-HT, 5-HIAA, and their ratio were basically consistent with previous findings. There was, however, a more robust response to this dose of the drug than was observed in the first experiment, so that females from all stages of the estrous cycle showed a significant decline in the 5-HIAA/5-HT ratio [ANOVA for drug treatment, $F(2, 48) = 21.15$, $p \leq 0.0001$; Dunnett's $q(48, 3) > 3.15$, $p \leq 0.05$]. Neither a significant effect of stage nor a significant stage by treatment interaction (ANOVA, $p > 0.05$) was present for the 5-HIAA/5-HT ratio.

However, a significant effect of stage was present for hypothalamic 5-HT, $F(2, 48) = 5.57$, $p \leq 0.007$, and 5-HIAA, $F(2, 48) = 4.005$, $p \leq 0.025$. In each case, the stage effect was due to higher levels of 5-HT and 5-HIAA in diestrous relative to estrous or proestrous females [respectively, Tukey $q(48, 3) = 4.07$ and $3.45$, $p \leq 0.05$]. An overall significant treatment effect was seen for 5-HIAA, $F(2, 48) = 9.28$, $p \leq 0.004$, but not for 5-HT, and the decline in 5-HIAA was significant only for the estrous females [Dunnett's $q(48, 3) = 3.2$, $p \leq 0.05$].

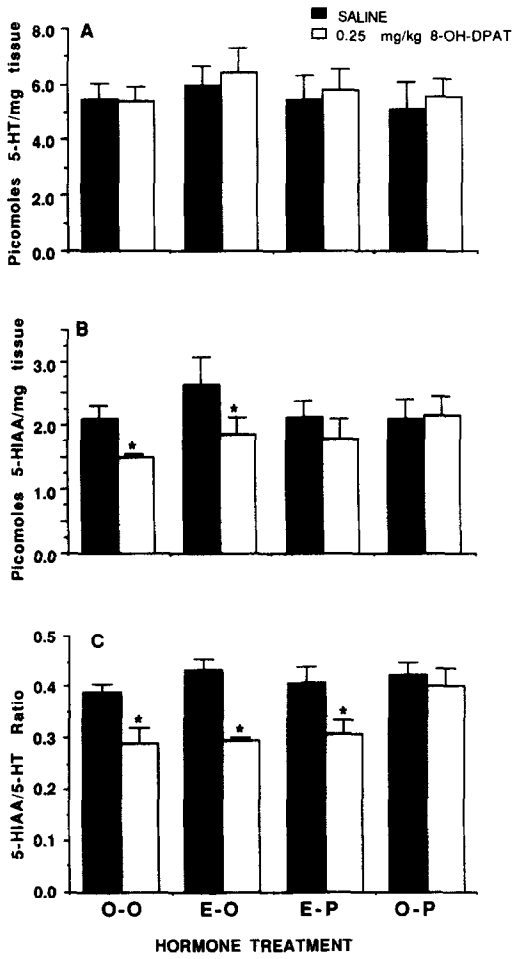


FIG. 3. Effects of 0.25 mg/kg 8-OH-DPAT on 5-HT and 5-HIAA and the 5-HIAA/5-HT ratio in the hypothalamus of hormone-primed ovariectomized rats. Ovariectomized rats were injected SC with oil or 25 μg estradiol benzoate. Forty-eight hours later, these rats received SC injections of either oil or 500 μg progesterone. These treatments resulted in four different groups: oil-oil (O-O), estradiol benzoate-oil (E-O), estradiol benzoate-progesterone (E-P), and oil-progesterone (O-P). Five to six hours after the second injection (progesterone or oil), these rats were injected IP with either saline or 0.25 mg/kg 8-OH-DPAT and decapitated 30 min later. Saline- and 8-OH-DPAT-injected rats were sacrificed in pairs. The hypothalamus was dissected and, as in Figs. 1 and 2, the hypothalamic levels of 5-HT, 5-HIAA, and the 5-HIAA/5-HT ratio were determined. The number of pairs for the O-O, E-O, E-P, and the O-P groups were 5, 6, 5, and 6, respectively. The mean ± SE pmol 5-HT and 5-HIAA per mg hypothalamus and their ratio are shown (A), (B), and (C), respectively. *Significant differences from the appropriate saline control.

TABLE 1

EFFECTS OF 0.25 mg/kg 8-OH-DPAT

| Stage | Treatment | 5-HT | 5-HIAA | 5-HIAA/5-HT Ratio |
|---|---|---|---|---|
| Hypothalmus | | | | |
| Proestrus | Saline | 4.85 ± 0.28 | 1.54 ± 0.28 | 0.31 ± 0.01 |
| | 8-OH-DPAT | 5.29 ± 0.31 | 1.35 ± 0.10 | 0.26 ± 0.20* |
| Diestrus | Saline | 5.94 ± 0.45 | 1.84 ± 0.13 | 0.32 ± 0.01 |
| | 8-OH-DPAT | 6.21 ± 0.40 | 1.58 ± 0.10 | 0.25 ± 0.01 |
| Estrus | Saline | 4.89 ± 0.17 | 1.60 ± 0.10 | 0.32 ± 0.01 |
| | 8-OH-DPAT | 5.23 ± 0.33 | 1.24 ± 0.09* | 0.25 ± 0.02* |
| Hippocampus | | | | |
| Proestrus | Saline | 3.00 ± 0.08 | 1.32 ± 0.05 | 0.44 ± 0.01 |
| | 8-OH-DPAT | 3.39 ± 0.08 | 1.24 ± 0.07 | 0.33 ± 0.02* |
| Diestrus | Saline | 2.93 ± 0.14 | 1.43 ± 0.10 | 0.48 ± 0.01 |
| | 8-OH-DPAT | 3.07 ± 0.11 | 1.27 ± 0.08 | 0.41 ± 0.02* |
| Estrus | Saline | 2.85 ± 0.14 | 1/30 ± 0.08 | 0.46 ± 0.02 |
| | 8-OH-DPAT | 3.32 ± 0.10 | 1.27 ± 0.07 | 0.38 ± 0.01* |

Values are mean ± SEM in pmole/mg tissue.

Proestrous, diestrous, or estrous female rats were treated IP with either saline or 0.25 mg/kg 8-OH-DPAT and decapitated 30 min later. The hypothalamus and the hippocampus were dissected and processed as described previously. The number of animals for the hypothalamic tissue in the saline and the 8-OH-DPAT groups were as follows: proestrus 10, 12; diestrus, 7, 8; and estrus, 8, 9. The numbers of animals were the same for the hippocampus in all except the saline-treated proestrus group, where a single sample was lost while processing.

*Significant differences from the appropriate saline control.

As is evident from Table 1, 8-OH-DPAT also decreased the 5-HIAA/5-HT ratio in hippocampal tissue, $F(2, 47) = 18.99, p \leq 0.0001$, and all 8-OH-DPAT-treated females were significantly different from their respective saline controls [Dunnett's $q(47, 3) = 5.93, p \leq 0.05$]. There was an increase in hippocampal 5-HT after treatment with 8-OH-DPAT, $F(2, 28) = 12.94, p \leq 0.0008$, but, unlike the hypothalamus, hippocampal levels of 5-HT did not vary with the estrous cycle (ANOVA, $p > 0.05$). Furthermore, there was only a small and nonsignificant decline in hippocampal 5-HIAA after treatment with 8-OH-DPAT (ANOVA for drug treatment, $p > 0.05$) and there was no evidence that effects of 8-OH-DPAT were estrous cycle dependent.

The data for the two tissues were normalized to their appropriate saline controls and the resulting ratios were used to directly compare the hippocampal and hypothalamic response to 8-OH-DPAT. As suggested above, the decline in hypothalamic 5-HIAA after treatment with 8-OH-DPAT was significantly greater than was the decline in hippocampal tissue, $F(1, 51) = 4.03, p \leq 0.05$. There were no significant differences in the two tissues for the 8-OH-DPAT-induced change in 5-HT or in the 5-HIAA/5-HT ratio.

## DISCUSSION

In general, these findings are consistent with previous observations for gender and estrous cycle differences in the response to 5-HT drugs. In addition, they support the suggestion that males and females differ in their response to $5\text{-HT}_{1A}$ agonists and that female gonadal hormones may contribute to this gender difference. Finally, in the present studies, relatively similar responses to 8-OH-DPAT were seen in both hippocampal and hypothalamic tissue; however, estrous cycle differences that were independent of the treatment with 8-OH-DPAT were present in hypothalamic, but not hippocampal, tissue.

In previous studies, males and diestrous females were shown to exhibit greater hyperphagia following treatment with 8-OH-DPAT than did estrous and proestrous females (41), and estrogen appeared to account for the differential sensitivity of the females to the hyperphagic effect of the drug (34). These findings were consistent with the report that estrogen reduced the effectiveness of 8-OH-DPAT in reducing firing of 5-HT neurons (28) and led us to suggest that changes in somatodendritic $5\text{-HT}_{1A}$ autoreceptor functioning were responsible for gender and estrous cycle differences in the hyperphagic response to 8-OH-DPAT. The present findings suggest that this hypothesis may have been oversimplified. In the present experiment, the change in the hypothalamic 5-HIAA/5-HT ratio following treatment with 8-OH-DPAT was most evident in diestrous and estrous females whereas males and proestrous females were less responsive. Furthermore, in ovariectomized rats, estrogen alone did not prevent the response to 8-OH-DPAT. In contrast, progesterone did attenuate the 8-OH-DPAT-induced decline in the 5-HIAA/5-HT ratio. Consequently, progesterone and/or the combination of estrogen plus progesterone may be required for the reduced sensitivity of the intact, proestrous rat to 8-OH-DPAT. Because progesterone levels of the estrous rat would be expected to exceed those of the proestrous rat, it is not clear why the response of estrous rats to 8-OH-DPAT was not more similar to that of the proestrous females.

Perhaps the most important finding to emerge from these studies is the differential effect of 8-OH-DPAT on 5-HT and 5-HIAA during the estrous cycle. Most investigators have assumed that activation of the $5\text{-HT}_{1A}$ autoreceptor and a consequent reduction in the release of 5-HT results in a decline in the 5-HIAA/5-HT ratio following treatment with 8-OH-

DPAT (21). Consequently, a decline in the 5-HIAA/5-HT ratio is often used as an index of the 5-$HT_{1A}$ agonist's effect on the somatodendritic autoreceptor (21,29). However, a change in the 5-HIAA/5-HT ratio can result from a change in synthesis, release, or degradation of 5-HT, leading to changes in either 5-HIAA, 5-HT, or both. Although a decrease in 5-HIAA would be expected to accompany a decrease in 5-HT release, some 5-HIAA may be derived from intraneuronal degradation of 5-HT prior to release (18,26) so that higher tissue levels of 5-HIAA could include unreleased 5-HT. Furthermore, because estrogen increases the reuptake of 5-HT into the nerve terminal (33) and progesterone attenuates this effect of estrogen (31), the relative contribution of released 5-HT to tissue levels of 5-HIAA may not be consistent across the estrous cycle. If, in fact, 8-OH-DPAT, by stimulating the 5-$HT_{1A}$ autoreceptor, reduces firing of 5-HT neurons and consequent release of 5-HT, the effects of 8-OH-DPAT on tissue levels of 5-HIAA should increase as a greater proportion of the 5-HIAA is derived from degradation of released and recycled 5-HT. Therefore, as observed in the current studies, (a) we would expect a greater effect of 8-OH-DPAT on tissue levels of 5-HIAA in ovariectomized rats treated only with estrogen relative to those given estrogen and progesterone; and (b) we would anticipate smaller effects of 8-OH-DPAT on tissue levels of 5-HIAA in the endogenously estrogen plus progesterone-primed proestrous rat. These results suggest cautious use of a decline in the 5-HIAA/5-HT ratio as an index of somatodendritic 5-$HT_{1A}$ autoreceptor function and reinforce the importance of examining the drug's effect independently on both 5-HT and 5-HIAA.

For example, the generally higher levels of 5-HIAA in saline-treated females compared to saline-treated males would be consistent with the suggestion that females are less subject to the autoinhibitory effects of 5-HT; in fact, an estrogen-induced desensitization of 5-$HT_{1A}$ autoreceptors could contribute to an increase in 5-HT release in females. In contrast, the overall greater response of females to the 5-$HT_{1A}$ agonist, 8-OH-DPAT, suggests that 5-$HT_{1A}$ autoreceptors in females continue to be responsive to the drug. However, the greater effectiveness of 8-OH-DPAT in females, relative to males, may also reflect the female's greater release and reuptake of 5-HT. In either case, it is unlikely that the presence of estrogen in females is solely responsible for the gender or estrous cycle differences in the response to 8-OH-DPAT. Moreover, it is unlikely that the mechanisms responsible for the estrous cycle differences are the same as those contributing to the gender differences in response to 8-OH-DPAT. Unfortunately, the contribution of male gonadal hormones to the gender difference was not investigated in the present study. However, it is clear that, in ovariectomized females, progesterone makes an important contribution to the differential response to 8-OH-DPAT. Although all mechanisms whereby progesterone modulates release and reuptake of 5-HT are not known, progesterone metabolites are thought to inhibit the firing of DR neurons by potentiating the inhibitory actions of GABA at the $GABA_A$ receptor (2,20). Consistent with the present findings, in progesterone-treated ovariectomized rats or in proestrous females, the relative contribution of the 5-$HT_{1A}$ somatodendritic autoreceptor to the regulation of DR firing should be decreased by the enhanced inhibitory contribution of GABA.

Thus, although female gonadal hormones clearly influence the effect of 8-OH-DPAT and the response to 8-OH-DPAT is estrous cycle dependent and gender specific, the explanation for these differences cannot reside simply in a differential sensitivity of 5-$HT_{1A}$ autoreceptors to the 5-$HT_{1A}$ agonist. It is increasingly evident that female gonadal hormones orchestrate the functioning of the 5-HT system at multiple neuroanatomical levels and that future work will be required to dissect the contribution of each level of control to the hormones' overall modulation of the 5-HT system.

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## REFERENCES

1. Ahlenius, S. N.; Fernandez-Gausti, A.; Hjorth, S.; Larsson, D. Suppression of lordosis behavior by the putative 5-HT receptor agonist, 8-OH-DPAT in the rat. Eur. J. Pharmacol. 124:361–363; 1986.
2. Barker, J. L.; Harrison, N. L.; Lange, G. D.; Majewska, M. D.; Owen, D. G. Voltage-clamp studies of the potentiation of GABA-activated chloride conductance by alphaxalone and a reduced metabolite of progesterone. J. Physiol. 372:48–58; 1986.
3. Biegon, A.; Bercovitz, H.; Samuel, D. Serotonin receptor concentration during the estrous cycle of the rat. Brain Res. 187:221–225; 1980.
4. Biegon, A.; Reches, A.; Snyder, L.; McEwen, B. S. Serotonergic and noradrenergic receptors in the rat brain: Modulation by chronic exposure to ovarian hormones. Life Sci. 32:2015–2021; 1983.
5. Blier, P.; DeMontigny, C.; Chaput, Y. Modifications of the serotonin system by antidepressant treatments: Implications for the therapeutic response in major depression. J. Clin. Psychopharmacol. 7:24S–35S; 1987.
6. Caldescoptt-Hazard, S.; Morgan, D. G.; DeLeon-Jones, F.; Overstreet, D. H.; Janowsky, D. Clinical and biochemical aspects of depressive disorders: II. Transmitter/receptor theories. Synapse 9:251–301; 1991.
7. Cohen, I. R.; Wise, P. M. Effects of estradiol on the diurnal rhythm of serotonin activity in microdissected brain areas of ovariectomized rats. Endocrinology 122:2619–2625; 1988.
8. Culter, M. G. An ethological study of the effects of buspirone and the 5-$HT_3$ receptor antagonist, BRL 43694 (Granisetron) on behavior during social interactions in female and male rats. Neuropharmacology 30:299–306; 1991.
9. DiPaolo, T.; Daigle, M.; Picard, V.; Barden, N. Effect of acute and chronic 17$\beta$-estradiol treatment on serotonin and 5-hydroxyindoleacetic acid content of discrete brain nuclei of ovariectomized rat. Exp. Brain Res. 51:73–76; 1983.
10. Fernandez-Guasti, A.; Picazo, O. The actions of diazepam and serotonergic anxiolytics vary according to the gender and the estrous cycle phase. Pharmacol. Biochem. Behav. 37:77–81; 1990.
11. Fishette, C. T.; Biegon, A.; McEwen, B. S. Sex steroid modulation of the serotonin behavioral syndrome. Life Sci. 35:1197–1206; 1984.
12. Fludder, J. M.; Tonge, S. R. Variations in the concentrations of monoamines and their metabolites in eight regions of rat brain during the estrous cycle: A basis for interactions between hormones and psychotropic drugs. J. Pharm. Pharmacol. 27:39P; 1975.
13. Frankfurt, M.; Renner, K.; Azmitia, E.; Luine, V. N. Intrahypo-

thalamic 5,7 dihydroxytryptamine: Temporal analysis of effects on 5-hydroxytryptamine content in brain nuclei and on facilitated lordosis behavior. Brain Res. 340:127–133; 1985
14. Gallager, D. W. Benzodiazepines: Potentiation of GABA inhibitory response in the dorsal raphe nucleus. Eur. J. Pharmacol. 49: 133–143; 1978.
15. Glennon, R. A. Serotonin receptors: Clinical implications. Neurosci. Biobehav. Rev. 14:35–47; 1990.
16. Glowinsky, J.; Iversen, L. L. Regional studies of catecholamines in rat brain. The disposition of $^3$H-norepinephrine, $^3$H-dopamine and $^3$H-dopa in various regions of the brain. J. Neurochem. 3: 665–669; 1966.
17. Graeff, F. G. Role of 5-HT in defensive behavior and anxiety. Rev. Neurosci. 4:181–211; 1993.
18. Grahame-Smith, D. G. How important is the synthesis of brain 5-hydroxytryptamine in the physiological control of its central function? Adv. Biochem. Psychopharmacol. 10:83–91; 1974.
19. Haleem, D. J.; Kennett, G. A.; Curzon.G. Hippocampal 5-hydroxytryptamine synthesis is greater in female rats than in males and more decreased by the 5-HT$_{1A}$ agonist 8-OH-DPAT. J. Neural Transm. 79:93–101; 1990.
20. Harrison, N. L.; Majewska, M. D.; Harrington, J. W.; Barker, J. L. Structure–activity relationships for steroid interaction with the gamma-aminobutyric acid receptor complex. J. Pharmacol. Exp. Ther. 241:346–353; 1987.
21. Hjorth, S. A.; Magnusson, T. The 5-HT$_{1A}$ receptor agonist, 8-OH-DPAT, preferentially activates cell body 5-HT autoreceptors in rat brain in vivo. Naunyn Schmiedebergs Arch. Pharmacol. 338:463–471; 1988.
22. Hjorth, S.; Sharp, T. Effect of the 5-HT$_{1A}$ receptor agonist on the release of 5-HT in dorsal and median raphe-innervated rat brain regions as measured by in vivo microdialysis. Life Sci. 48: 1779–1786; 1991.
23. Kennett, G. A.; Chaouloff, F.; Marcou, M.; Curzon, G. Female rats are more vulnerable than males in an animal model of depression, the possible role of serotonin. Brain Res. 382:416–421; 1986.
24. Kennett, G. A.; Dourish, C. T.; Curzon, G. Antidepressant-like action of 5-HT$_{1A}$ agonists and conventional antidepressants in an animal model of depression. Eur. J. Pharmacol. 134:265–274; 1987.
25. Kilts, C. D.; Breese, G. R.; Mailman, R. B. Simultaneous quantification of dopamine, 5-hydroxytryptamine and four metabolically related compounds by means of reversed-phase liquid chromatography with electrochemical detection. Chromatography 223:347–357; 1981.
26. Kuhn, D. M.; Wolf, W. A.; Youdim, M. B. H. Serotonin neurochemistry revisited: A new look at some old axioms. Neurochem. Int. 8:141–154; 1974.
27. Ladisich, W. Effect of progesterone on regional 5-hydroxytryptamine metabolism in the rat brain. Neuropharmacology 13: 877–883; 1974.
28. Lakoski, J. L. Estrogen-induced modulation of serotonin 5-HT$_{1A}$ mediated responses in the dorsal raphe nucleus (DRN). Pharmacologist 30:A126–128; 1988.
29. Larsson, L.-G.; Renyi, L.; Ross, S. B.; Svensson, B.; Angeby-Moller, K. Different effects on the responses of functional preand postsynaptic 5-HT$_{1A}$ receptors by repeated treatment of rats with the 5-HT$_{1A}$ receptor agonist 8-OH-DPAT. Neuropharmacology 29:85–91; 1991.
30. Lesch, K. P.; Mayer, S.; Disselkamp-Tietze, J.; Hoh, A.; Weismann, M.; Osterheider, M.; Schulte, H. M. 5-HT$_{1A}$ receptor responsivity in unipolar depression. Evaluation of ipsapirone-induced ACTH and cortisol secretion in patients and controls. Biol. Psychiatry 28:620–628; 1990.
31. Meyerson, B. J; Malmnas, C. O.; Everitt, B. J. Neuropharmacology, neurotransmitters and sexual behavior in mammals. In: Adler, N.; Pfaff, D.; Goy, R. W., eds. Handbook of behavioral neurobiology. New York: Plenum Press; 1985:495–536.
32. Rausch, J. L.; Ruegg, R.; Moeller, F. G. Gepirone as a 5-HT$_{1A}$ agonist in the treatment of major depression. Psychopharmacol. Bull. 26:169–171; 1990.
33. Rehavi, M.; Sepcuti, H.; Weizman, A. Upregulation of imipramine binding and serotonin uptake by estradiol in female rat brain. Brain Res. 410:135–139; 1987.
34. Salamanca, S.; Uphouse, L. Estradiol modulation of the hyperphagia induced by the 5-HT$_{1A}$ agonist, 8-OH-DPAT. Pharmacol. Biochem. Behav. 43:953–955; 1992.
35. Sharp, T.; Bramwell, S. R.; Grahame-Smith, D. G. 5-HT$_1$ agonists reduce 5-hydroxytryptamine release in rat hippocampus in vivo as determined by brain microdialysis. Br. J. Pharmacol. 96: 283–290; 1989.
36. Smith, S. S.; Waterhouse, B. D.; Chapin, J. K.; Woodward, D. J. Progesterone alters GABA and glutamate responsiveness: A possible mechanism for its anxiolytic action. Brain Res. 400:353–359; 1987.
37. Sprouse, H. S.; Aghajanian, G. K. Electrophysiological responses of serotonergic dorsal raphe neurons to 5-HT$_{1A}$ and 5-HT$_{1B}$ agonists. Synapse 1:3–9; 1987.
38. Trulson, M. E.; Preussler, D. W.; Howell, G. A.; Fredericson, C. J. Raphe unit activity in freely moving cats: Effects of benzodiazepines. Neuropharmacology 21:1045–1050; 1982.
39. Uphouse, L.; Caldarola-Pastuszka, M.; Montanez, S. Intracerebral actions of the 5-HT$_{1A}$ agonists, 8-OH-DPAT and buspirone, and of the 5-HT$_{1A}$ partial agonist/antagonist, NAN-190, on female sexual behaviors. Neuropharmacology 31:969–981; 1992.
40. Uphouse, L.; Montanez, S.; Richards-Hills, R.; Caldarola-Pastuszka, M.; Droge, M. Effects of 8-OH-DPAT on sexual behaviors of the proestrous rat. Pharmacol. Biochem. Behav. 39: 635–640; 1991.
41. Uphouse, L.; Salamanca, S.; Caldarola-Pastuszka, M. Gender and estrous cycle differences in the response to the 5-HT$_{1A}$ agonist 8-OH-DPAT. Pharmacol. Biochem. Behav. 40:901–906; 1991.
42. Uphouse, L.; Williams, J.; Eckols, K.; Sierra, V. Variations in binding of $^3$H-5-HT to cortical membranes during the female estrous cycle. Brain Res. 381:376–381; 1986.
43. Wilkinson, L. O.; Dourish, C. T. Serotonin and animal behavior. In: Peroutka, S. J., ed. Serotonin receptor subtypes. Basic and clinical aspects. New York: Wiley-Liss, Inc.; 1991:147–210.
44. Williams, J.; Uphouse, L. Serotonin binding sites during proestrus and following estradiol treatment. Pharmacol. Biochem. Behav. 33: 615–620; 1990.
46. Zar, J. H. Biostatistical analysis, second edition. Englewood Cliffs, NJ: Prentice Hall, Inc.; 1984.