

SEROTONERGIC STEREOTYPY AND SEXUAL BEHAVIOR IN THE RAT:
REGULATION BY CORTICOSTERONE.

by

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ABSTRACT

Both adrenalectomy and the adrenal hormone corticosterone have been shown to alter serotonergic type 2A (5-HT_{2A}) receptor activity. 5-HT_{2A} receptors mediate effects on both "wet dog shakes" (WDS) and sexual behavior in the rat. In the present series of experiments, the potential involvement of corticosterone in the regulation of WDS and sexual behavior in the male and female rat was investigated. In Experiment 1, bilaterally-adrenalectomized rats were compared to sham-adrenalectomized rats in the frequency of both spontaneous WDS, and WDS following the administration of the 5-HT_{2A} agonist DOI. Adrenalectomy resulted in a significant reduction of DOI-induced WDS in both the male and female rat. In Experiment 2, rats chronically received either corticosterone or oil and were again scored for WDS behavior. Following DOI treatment, corticosterone effectively blocked the adrenalectomy-induced reduction of WDS in both the male and female rat. In Experiment 3, adrenally-intact male rats received either chronic corticosterone injections or oil injections. Rats were then compared on measures of both spontaneous and DOI-induced WDS and sexual behavior. Rats receiving chronic corticosterone exhibited a significant increase in WDS and a marked inhibition of male sexual behavior in both the spontaneous and DOI conditions. In Experiment 4, both adrenalectomized and sham-adrenalectomized females were divided into groups receiving either chronic corticosterone or oil. Rats were compared on measures of spontaneous and DOI-induced WDS and sexual behavior following the administration of estrogen alone, or estrogen in combination with progesterone. Chronic corticosterone administration increased WDS and facilitated female sexual behavior, while adrenalectomy decreased WDS and had mixed effects on female sexual behavior. These results suggest that the adrenal steroid corticosterone is important in the regulation of WDS and sexual behavior in both the male and female rat, and that this regulation may be mediated by activity at 5-HT_{2A} receptors.

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INTRODUCTION

The past decade has seen major advances in the understanding of serotonin's (5-HT) role in various aspects of behavior. These recent developments in the behavioral pharmacology of 5-HT can be attributed to the identification of multiple 5-HT receptors and the increasing availability of selective drugs that act on the range of 5-HT receptors. To date, the literature has described at least ten 5-HT binding sites, and the present system of classification involves the differentiation of 5-HT receptors into four major families: 5-HT₁, 5-HT₂, 5-HT₃ and 5-HT₄ (Hoyer & Martin, 1996). Within both the 5HT₁ and 5HT₂ families exist a number of functional receptor subtypes. For example, the 5-HT₂ family consists of three distinct subtypes: 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} (Hoyer & Martin, 1996).

Studies employing 5-HT agonists and antagonists selective for specific receptors have demonstrated the behavioral complexity of the serotonergic system. It is now known that specific 5-HT receptors can mediate opposing effects on the same behavior. For example, sexual behavior can be either inhibited or facilitated depending on which 5-HT receptor subtype is activated (Gorzalka, Mendelson & Watson, 1990). It has also been demonstrated that specific 5-HT receptor subtypes may mediate differential effects on female and male rat sexual behavior (Mendelson & Gorzalka, 1986a; Gorzalka, et al, 1990). In particular, the 5-HT_{2A} receptor has been shown to have different effects on the expression of sexual behavior depending on the sex of the animal. In the female rat, administration of relatively selective 5-HT_{2A} agonists, such as 1-(2,5-dimethoxyphenyl-4-iodo)-2-aminopropane (DOI), have been shown to stimulate lordosis behavior, the primary indicator of sexual receptivity in females (James, Lane, Hole & Wilson, 1989), while relatively selective 5-HT_{2A} antagonists, such as pirenperone and ketanserin, inhibit lordosis behavior (Mendelson & Gorzalka, 1986b; Mendelson & Gorzalka, 1985a). Taken together, these findings support the suggestion that 5-HT_{2A} activation in the female rat facilitates sexual behavior (Mendelson & Gorzalka, 1989). However, in the male rat, the effects

of 5-HT_{2A} antagonists on sexual behavior are opposite to the effects seen in the female. After administration of 5-HT_{2A} antagonists, pirenperone or ketanserin, sexual behavior in both naive and sexually vigorous males was facilitated, as evidenced by significantly fewer mounts and intromissions prior to ejaculation, and shorter latencies and post-ejaculatory intervals (Mendelson & Gorzalka, 1985b). The administration of the 5-HT_{2A} agonist, DOI, inhibited sexual behavior, and this effect was blocked by 5-HT_{2A} antagonists (Watson & Gorzalka, 1991). Overall, the available data suggest that 5-HT_{2A} receptor activity mediates a facilitation of female sexual behavior and an inhibition of male sexual behavior.

Prior to the discovery of multiple 5-HT receptors, "wet dog shakes" (WDS) were identified and described in the rat. WDS, a quivering shudder of the head, neck, and trunk, were found to increase in frequency with increases in 5-HT activity (Bedard & Pycock, 1977). Once multiple 5-HT receptors were identified and studies began to employ selective drugs, it was concluded that WDS are primarily mediated by 5-HT_{2A} activity (Yap & Taylor, 1983). WDS can be induced pharmacologically with 5-HT_{2A} agonists in rats and mice (Goodwin, Green & Johnson, 1984; Yap & Taylor, 1983), and have since been used as a behavioral assay for quantifying 5-HT_{2A} activity (Watson & Gorzalka, 1990). Central administration of DOI produces a dose-dependent increase in WDS which is attenuated by the administration of the 5-HT_{2A} antagonist, ritanserin (Watson & Gorzalka, 1992). Because DOI has high affinity for both 5-HT_{2A} and 5-HT_{2C} receptors (Hoyer, 1988), it is important to rule out the possibility that DOI influences WDS via 5-HT_{2C} activity. There is evidence to suggest that 5-HT_{2C} receptors are unlikely to be involved in eliciting WDS. For example, metergoline and spiperone, two 5-HT_{2A} antagonists, were equipotent at blocking DOI-induced WDS even though metergoline has a much greater affinity for the 5-HT_{2C} receptor than spiperone (Skingle, Malcolm, Cole & Nicola, 1991). Therefore, the action of DOI on 5-HT_{2C} receptors is not likely relevant to the display of DOI-induced WDS.

Spontaneously occurring WDS (as opposed to DOI-induced WDS) have been used as a noninvasive measure of endogenous 5-HT_{2A} activity occurring concurrently with sexual behavior in the male rat (Watson & Gorzalka, 1990). It was shown that the frequency of copulatory behavior was inversely correlated with the frequency of WDS. That is, vigorous copulators showed few WDS, noncopulators showed considerable WDS, and moderate copulators were intermediate. DOI enhanced WDS and inhibited ejaculations. These results supported the hypothesis that increased 5-HT_{2A} activity mediates a facilitation in WDS behavior and an inhibition in sexual behavior in the male rat. To date, there have been no reports in the literature on the relationship between WDS and sexual behavior in the female rat. However, based on pharmacological studies which suggest that female sexual behavior is also 5-HT_{2A}-mediated, it is expected that a facilitation in female sexual behavior after administration of a 5-HT_{2A} would be accompanied by an increase in WDS; and like in the male rat, spontaneously occurring WDS can be used as an *in vivo* measure of 5-HT_{2A} activity.

Research has demonstrated strong interactions between hormones of the hypothalamic-pituitary-adrenal (HPA) axis and the central serotonergic systems: Glucocorticoid receptors are present on almost all 5-HT neurons, blood levels of HPA axis hormones affect 5-HT neurotransmission and, conversely, 5-HT transmission alters the blood levels of HPA hormones (Chaouloff, 1993). Since the discovery of multiple serotonin receptors, research has revealed that the HPA axis interacts with specific 5-HT receptors. The interactions between the HPA axis and 5-HT_{2A} receptor activity is of considerable interest because of its clinical significance: It has been suggested that the proximate cause of major depression is a deficiency in 5-HT function (Koyama & Meltzer, 1986), and of the receptor subtypes, 5-HT_{2A} has been most closely associated to depression. Reports have shown an increase in 5-HT_{2A} binding sites in the brains of suicide victims and patients with depression (Mann, Stanley, McBride, & McEwen, 1986; Yates, Leake, Candy, Fairbairn, McKeith & Ferrier, 1990). Among the latter, chronic antidepressant treatment has the effect of decreasing the number of 5-HT_{2A} binding sites

(Peroutka & Snyder, 1980a, 1980b). The increase in 5-HT_{2A} binding sites seen in depressives is not well understood. 5-HT_{2A} receptors appear to be relatively resistant to upregulation; it has been shown that most pre- and post-synaptic manipulations fail to promote an upregulation (Sanders-Bush, 1990). This suggests the possibility that non-serotonergic systems play a role in the regulation of 5-HT_{2A} receptor density - in particular, there is evidence suggesting that hormones of the HPA axis may influence this regulation.

The relationship between the HPA axis and 5-HT_{2A} receptor activity is not fully understood. Nevertheless, the available evidence suggests that chronically elevated levels of corticosterone result in an upregulation of 5-HT_{2A} receptor density in selective regions of the brain. Pharmacological and physiological studies have demonstrated a positive association between 5-HT_{2A} activity and blood levels of corticosterone (Alper, 1990; Calogero, Bagdy, Szemerédi, Tartaglia, Gold & Chrousos, 1990). Radioligand binding studies have shown that adrenalectomy causes region-specific changes in 5-HT_{2A} receptor density (Martire, Pistritto & Preziosi, 1989; Kuroda, Mikuni, Ogawa & Takahashi, 1992), and that chronic administration of corticosterone increases 5-HT_{2A} binding in the neocortex (Kuroda, et al, 1992). Chronic social stress has been shown to increase 5-HT_{2A} binding in the parietal cortex - presumably by acting to elevate corticosterone levels (McKittrick, Blanchard, Blanchard, McEwen & Sakai, 1995). Studies investigating the influence of HPA axis hormones on 5-HT_{2A}-mediated behaviors are limited, and their results contradictory.

The only two studies which have examined the effect of HPA disruption (by adrenalectomy) on WDS have yielded contradictory results. One study reported that adrenalectomy significantly reduced the WDS behavior seen following the administration of the 5-HT_{2A} agonist DOI (Kuroda, et al, 1992), whereas another study reported that adrenalectomy did not affect 5-HT_{2A} receptor-mediated head shakes following the administration of DOI (Chaouloff, Baudrie & Coupry, 1993).

The role of the HPA axis in the regulation of sexual behavior is not clearly defined. The limited data available suggest that the HPA axis may exert differential effects on male and female rat sexual behavior. Psychosocial stress dramatically increases sexual receptivity and proceptivity in ovariectomized female rats primed with estrogen (Williams, McGinnis & Lumia, 1992). The facilitation in sexual receptivity has also been observed in ovariectomized, but not ovariectomized-adrenalectomized, females subjected to chronic individual housing (Gorzalka & Raible, 1981). These effects can be explained in the context of the imposed housing conditions acting as a stressor to increase adrenocortical secretions which then facilitated receptivity. In the male, chronic sequential exposure to a variety of mild, unpredictable stressors produced opposite effects, with a significant decrease in sexual behavior, evidenced by a higher frequency of mounts and an almost complete disruption of intromissions (D'Aquila, Brain & Willner, 1994; Sato, Kumamoto & Suzuki, 1992). Postpubertal individual housing of the male rat, acts as a stressor as it does in the female, and has been shown to significantly inhibit certain components of the male sexual response pattern (deCatanzaro & Gorzalka, 1979). In each of these situations, there was a dramatic elevation in serum corticosterone (the major adrenal glucocorticoid that is secreted in response to activation of the HPA axis). Studies which have directly examined the effects of corticosterone on sexual behavior have provided contradictory results. In the female rat, acute administration of corticosterone has been reported to both increase (Plas-Roser & Aron, 1981) and to not affect (Gorzalka & Whalen, 1977) sexual receptivity, whereas chronic administration of corticosterone has been shown to inhibit lordosis (deCatanzaro, Knipping & Gorzalka, 1981). To the best of our knowledge, no studies have directly examined the effects of postpubertal corticosterone administration on sexual behavior in the male rat. Adrenalectomy has been reported to facilitate female sexual behavior in rats treated with chronic estrogen (Gorzalka & Raible, 1981), however, the majority of studies in which adrenalectomized female rats were given acute estrogen have failed to find an effect on sexual behavior (Kow & Pfaff, 1975; Larsson, Feder & Komisaruk, 1974). Short-term

adrenalectomy appears to have no effect on male sexual behavior (Vega-Matuszczyk, Ahlenius, Eneroth & Larsson, 1995; Poggioli et al., 1984), however long-term adrenalectomy may prevent the age-linked decline in male sexual activity (Poggioli et al., 1984). In summary, adrenocortical secretions appear to have differential effects on male and female sexual behavior. Since 5-HT_{2A} activation also has differential effects on male and female sexual behavior, the possibility exists that 5-HT_{2A} receptors mediate the effects of adrenocortical steroids on sexual behavior. To date, this possibility has not been investigated.

Therefore, the purpose of the present investigation was to further clarify the HPA-5-HT_{2A} relationship using WDS as a measure of 5-HT_{2A} activity, and to examine the influence of chronic corticosterone on both male and female sexual behavior.

EXPERIMENT 1

The first experiment was designed to examine the effect of adrenalectomy on 5-HT_{2A} activity using WDS as a behavioral index. Since spontaneous WDS occur with a relatively low frequency, WDS were also observed after the administration of the 5-HT_{2A} agonist DOI. Therefore, in the present experiment both male and female adrenalectomized rats were compared to sham-adrenalectomized rats on measures of both spontaneous and DOI-induced WDS. The two studies which have investigated the effects of adrenalectomy on WDS in the male rat have yielded contradictory results (Kuroda et al., 1992; Chaouloff et al., 1993) and to date, no study has investigated the effects of adrenalectomy on WDS in the female rat.

Experiment 1A

Animals

Twenty male Wistar rats were obtained from Charles River Canada Inc., Montreal, at 60 days of age. Rats were housed in groups of three in triple wire mesh cages. The animals were allowed free access to Purina Rat Chow and water, and were maintained on a reversed 12/12 hr light/dark cycle with lights off at 0900h.

Surgery

At approximately 8 months of age (400-500g), rats received either bilateral adrenalectomy (ADX; n=10) or sham adrenalectomy (SHAM; n=10). Animals were anesthetized with intraperitoneal (IP) injection of sodium pentobarbital (65 mg/kg) prior to surgery, and all surgeries were performed via bilateral lumbar incisions. Sham-adrenalectomy involved all the same surgical procedures as adrenalectomy, with the exception that the adrenals were not removed. Following surgery, ADX rats were maintained on a 0.9% saline solution and 10% sucrose solution for three days, during which no solid food was available. After three days, the ADX rats were rehoused and maintained on 0.9% saline solution while given free access to food. The SHAM rats were returned to their prior living conditions as soon as they had recovered from surgery.

Behavioral Testing

Behavioral testing began 3 weeks following surgery. All behavioral testing was carried out during the middle third of the dark cycle, three weeks after the surgical procedure. All rats were tested for both spontaneous WDS and DOI-induced WDS. Thirty minutes before testing for

spontaneous WDS behaviour, all rats were subcutaneously (SC) injected with 0.9% saline solution (1 ml/kg). Each rat was tested for WDS in individual Plexiglas testing chambers (30x30x45 cm in height). Testing was performed by a trained observer who remained blind to the surgical condition of the rats. The rats were allowed a five minute habituation period before being scored for WDS over a thirty minute period. After all rats were tested for spontaneous WDS, they were then scored for WDS following the administration of DOI. (\pm) DOI hydrochloride (Research Biochemicals, Natick, MA) was dissolved in 0.9% saline solution (1 mg/ml) and a dose of 1 mg/kg was injected SC 30 minutes prior to behavioral testing. A 30 minute delay between injection and testing was imposed, because data from our laboratory have indicated that the maximum effect of DOI (1 mg/kg) on WDS occurs between 30 and 120 minutes following its administration. DOI-induced WDS was scored in the same manner as for spontaneous WDS.

Data were analyzed separately for both the spontaneous and DOI conditions using an independent samples t-test with a Bonferroni-adjusted alpha level of 0.025. Levene's test for equality of variance was performed on each variable prior to performing t-test analyses. If the results of Levene's test were found to be statistically significant ($p < 0.05$), indicating heterogenous variances, a Welch's correction to the degrees of freedom was performed in order to maintain control over Type I errors.

Results and Discussion

Data for spontaneous and DOI-induced WDS are presented in Table 1. Adrenalectomy had no significant effect on the number of spontaneous WDS displayed ($t(18) = 0.60, p = 0.56$). However, following the administration of DOI, adrenalectomy significantly reduced WDS ($t(18) = 5.17, p = 0.001$).

These results are consistent with those of Kuroda and colleagues (1992), who reported that disruption of the HPA axis by adrenalectomy results in a reduction of DOI-induced WDS. By using the frequency of WDS as a behavioral index of 5-HT_{2A} activity, these results suggest that adrenalectomy has the effect of decreasing activity at 5-HT_{2A} receptors. The lack of significant differences found in the spontaneous condition may be explained by the low frequency of spontaneous WDS displayed by both groups - only three rats showed any WDS behavior in the 30-minute test period.

TABLE 1
EFFECTS OF ADRENALECTOMY ON SPONTANEOUS
AND DOI-INDUCED WDS (MEAN±SEM) IN THE MALE RAT

	SHAM	ADX
Spontaneous	0.20 ± 0.13	0.10 ± 0.10
DOI-Induced	7.50 ± 0.86	1.90 ± 0.66

Experiment 1B

Animals

Thirty-two female Wistar rats were obtained from Charles River Canada Inc., Montreal, at 60 days of age. Rats were housed in groups of 3 in triple wire mesh cages. The animals were allowed free access to Purina Rat Chow and water, and were maintained on a reversed 12/12 hr light/dark cycle with lights off at 0900h.

Surgery

At two months of age (180-220g), all females were bilaterally ovariectomized while under a combination of sodium pentobarbital (50 mg/kg IP) and ketamine (0.5 mg/kg IP) anesthesia.

At approximately 3 months of age (250-300g), rats received either bilateral adrenalectomy (ADX; n=16) or sham-adrenalectomy (SHAM; n=16). Except for the anesthetic regimen (sodium pentobarbital (50 mg/kg IP) and ketamine (0.50 mg/kg IP)), both surgical and post-operative care were identical to procedures outlined in Experiment 1A.

Behavioral Testing

Behavioral testing procedures and subsequent statistical analyses were identical to those outlined in Experiment 1A.

Results and Discussion

Data for both spontaneous and DOI-induced WDS are presented in Table 2. As in the males, adrenalectomy was found to have no significant effect on the display of spontaneous WDS by female rats ($t(30) = 0.44$, $p = 0.67$). However, following the administration of DOI, adrenalectomy significantly reduced WDS ($t(30) = 6.52$, $p = 0.001$).

The effects of adrenalectomy on spontaneous and DOI-induced WDS in the female rat are consistent with those observed in the male rat. The effects of adrenalectomy on spontaneous WDS were not apparent possibly due to the low frequency with which spontaneous WDS occur.

TABLE 2
EFFECTS OF ADRENALECTOMY ON SPONTANEOUS
AND DOI-INDUCED WDS (MEAN±SEM) IN THE FEMALE RAT

	SHAM	ADX
Spontaneous	1.69 ± 0.49	1.38 ± 0.52
DOI-Induced	20.56 ± 1.55	8.38 ± 1.04

A comparison of the data obtained from both Experiments 1A and 1B using two-way ANOVA (surgery x gender) reveals that the females displayed significantly more spontaneous WDS ($F(1,48) = 8.97, p = 0.004$) and DOI-induced WDS ($F(1,48) = 59.78, p = 0.0001$) than the males. This is the first study to report a sex difference in the display of WDS. Recent evidence has suggested that 5-HT_{2A} receptor activity may influence behavior through a neural system that is modulated by testosterone (Padoin & Lucion, 1995; Gonzalez, Farabollini, Albonetti, & Wilson, 1994). It is possible that the presence of testosterone may act to suppress WDS behavior.

Taken together, results from Experiment 1A and 1B suggest that adrenalectomy inhibits the display of DOI-induced WDS. Radioligand binding studies which have examined the effects of adrenalectomy on 5-HT_{2A} receptor density are limited and have provided mixed results. Adrenalectomy has been reported to have no effect on 5-HT_{2A} binding in the neocortex (Kuroda et al., 1992; Chaouloff et al., 1993) or hypothalamus (Martire et al., 1989) but elicited an upregulation in the hippocampus (Martire et al., 1989). The present study replicates results which show an adrenalectomy-related decrease in 5-HT_{2A}-mediated WDS (Kuroda et al., 1992) suggesting a downregulation in 5-HT_{2A} receptor density. Given that the effects of

adrenalectomy on 5-HT_{2A} receptor density appear to have a regional specificity, it remains possible that adrenalectomy downregulates 5-HT_{2A} receptors in the brain regions involved in the generation of WDS. WDS has been shown to be unaffected by both total ablation of the frontal cortex (Lucki & Minugh-Purvis, 1987) and intrathecal injection of the serotonergic neurotoxin 5,7-dihydroxytryptamine (Fone, Johnson, Bennett & Marsden, 1989), suggesting that WDS originates somewhere in either the brainstem or the midbrain. To date, no studies have examined the effects of adrenalectomy on 5-HT_{2A} receptor binding in these brain regions.

EXPERIMENT 2

Experiment 1 provided evidence that adrenalectomy leads to a reduction in the 5-HT_{2A}-mediated behavior, WDS. These results suggest that adrenal steroid output causes an increase in 5-HT_{2A} activity. It has previously been shown that chronic administration of adrenocorticotrophic hormone (ACTH) significantly increases WDS in nonadrenalectomized animals and that chronic administration of corticosterone (both 20 and 50 mg/kg administered for 10 days) results in a significant increase in the density of 5-HT_{2A} receptor binding sites in the neocortex of the rat (Kuroda et al., 1992). Therefore, it is possible that the reduction in WDS seen in adrenalectomized rats in Experiment 1 is related to the decrease in corticosterone levels. Experiment 2 was designed to determine if chronic corticosterone treatment could reverse the adrenalectomy-induced reduction of WDS in both the male and female rat.

Experiment 2A

Animals

This experiment used the same 10 ADX and 10 SHAM male rats as were employed in Experiment 1A. Males were approximately 10 months of age when testing commenced.

Procedure

Half of the adrenalectomized rats were randomly chosen to receive corticosterone chronically (ADX-CORT n=5), while the other half received oil chronically (ADX-OIL n=5). The sham-adrenalectomized rats were divided similarly (SHAM-CORT n=5; SHAM-OIL n=5). Corticosterone-21-acetate (Sigma, St. Louis, MO) was suspended in sesame oil (50 mg/ml) and was injected subcutaneously daily for 10 days (1 ml/kg). Control animals received sesame oil (1 ml/kg) for the same duration. All behavioral testing occurred on the 11th day. Both spontaneous and DOI-induced WDS were observed using the same procedure as in Experiment 1.

Data were analyzed separately for the spontaneous and DOI conditions using two-way analyses of variance (ANOVA) with a Bonferroni-adjusted alpha level of 0.025 and pairwise comparisons using the Newman-Keuls test.

Results and Discussion

All data for spontaneous WDS are given in Table 3. No significant differences in the frequency of WDS were obtained in the spontaneous condition (surgery $F(1,16) = 0.10$, $p = 0.76$; treatment $F(1,16) = 2.38$, $p = 0.14$); this is not unexpected since the majority of rats showed no spontaneous WDS behavior during the 30-minute observation period.

TABLE 3

EFFECTS OF CHRONIC CORTICOSTERONE AND ADRENALECTOMY ON SPONTANEOUS WDS (MEAN \pm SEM) IN THE MALE RAT

	SHAM	ADX
OIL	0.40 \pm 0.24	0.60 \pm 0.40
CORT	1.00 \pm 0.00	1.06 \pm 0.45

Data obtained from the DOI condition are presented in Figure 1. Analysis of variance of DOI-induced WDS revealed a significant main effect of corticosterone treatment ($F(1,16) = 44.23, p = 0.001$) and a significant main effect of surgery ($F(1,16) = 7.25, p = 0.02$).

Subsequent pairwise comparisons revealed that all groups differed significantly from each other with the exception of ADX-CORT and SHAM-CORT.

As in Experiment 1, the adrenalectomized rats showed significantly fewer WDS than the sham-adrenalectomized rats in the absence of corticosterone treatment. Chronic corticosterone treatment had the effect of increasing the frequency of DOI-induced WDS regardless of the surgical condition of the animals. Corticosterone not only effectively blocked the adrenalectomy-induced reduction of WDS but also significantly increased the frequency of WDS in intact animals. These results suggest that chronically elevated levels of corticosterone may lead to an increase in 5-HT_{2A} receptor activity.

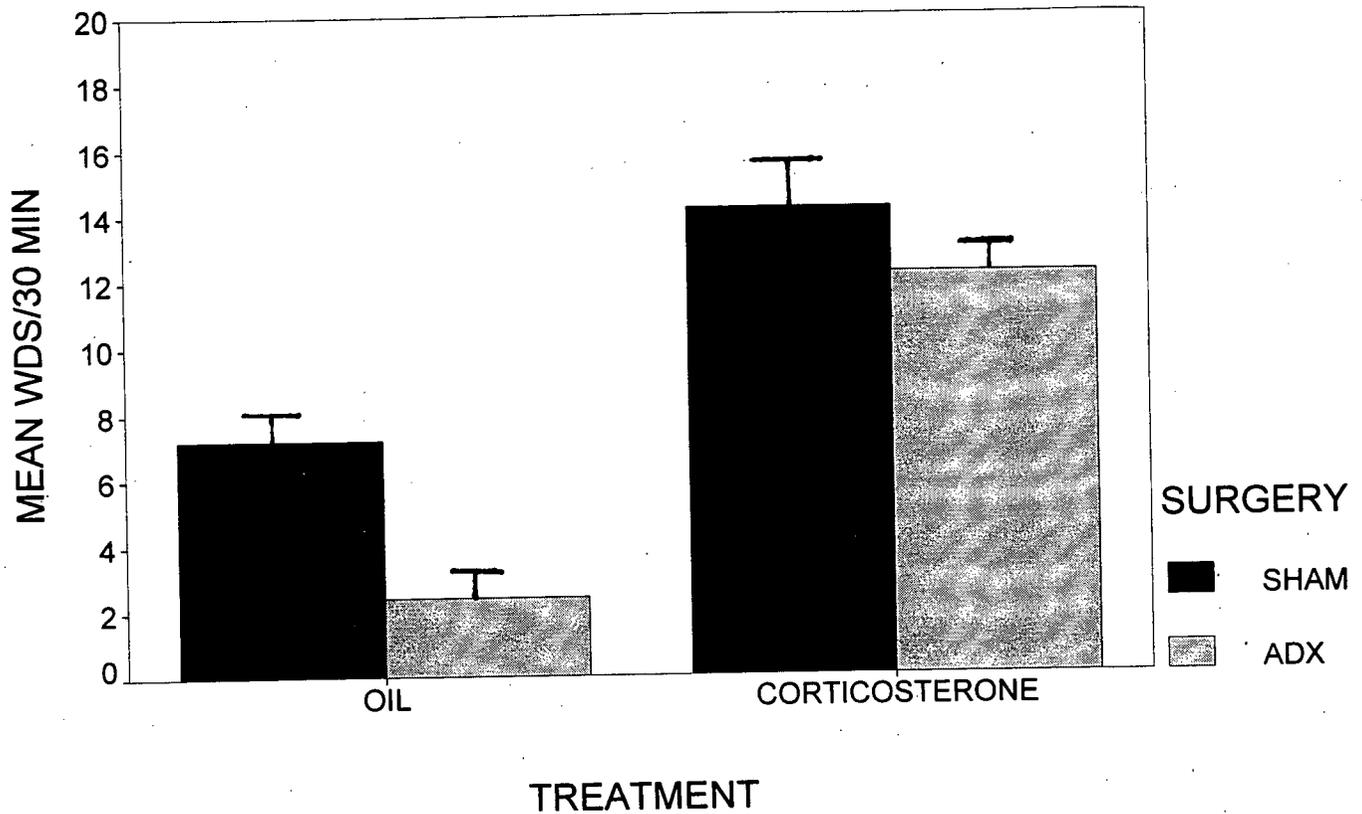


Figure 1: Effects of Chronic Corticosterone on DOI-Induced WDS in the Sham-Adrenalectomized (SHAM) and Adrenalectomized (ADX) Male Rat.

Experiment 2B

Animals

This experiment used the same 16 ADX and 16 SHAM female rats employed in Experiment 1B. Females were 5 months old at the time of testing.

Procedure

Half of the adrenalectomized rats were randomly chosen to receive corticosterone chronically (ADX-CORT n=8), while the other half received propylene glycol chronically (ADX-PROP n=8). The sham-adrenalectomized rats were divided similarly (SHAM-CORT n=8; SHAM-PROP n=8). Corticosterone-21-acetate (Sigma) was suspended in propylene glycol (50 mg/ml) and was injected subcutaneously daily for 10 days (1 ml/kg). Control animals received propylene glycol (1 ml/kg) for the same duration. All behavioral testing occurred on the 11th day. Both behavioral testing and statistical analyses were performed according to the same procedure described in Experiment 2A.

Results and Discussion

Data for spontaneous WDS are presented in Table 4. No significant differences were found in the spontaneous condition (surgery $F(1,28) = 0.13$, $p = 0.72$; treatment $F(1,28) = 1.19$, $p = 0.29$) which likely reflects the low frequency with which spontaneous WDS occurs.

TABLE 4
EFFECTS OF CHRONIC CORTICOSTERONE AND ADRENALECTOMY ON
SPONTANEOUS WDS (MEAN±SEM) IN THE FEMALE RAT

	SHAM	ADX
OIL	0.63 ± 0.18	0.88 ± 0.30
CORT	1.13 ± 0.48	1.22 ± 0.35

Data obtained from the DOI condition are presented in Figure 2. Analysis of variance of DOI-induced WDS revealed significant main effects for both corticosterone treatment ($F(1,28) = 40.55, p = 0.001$) and surgical condition ($F(1,28) = 9.96, p = 0.004$). Pairwise comparisons showed that all groups differed significantly from each other. The same trends reported in the male rat (Expt. 2A) were seen for the female rat. Chronic corticosterone reversed the adrenalectomy-induced reduction of WDS as well as increased the WDS of intact females.

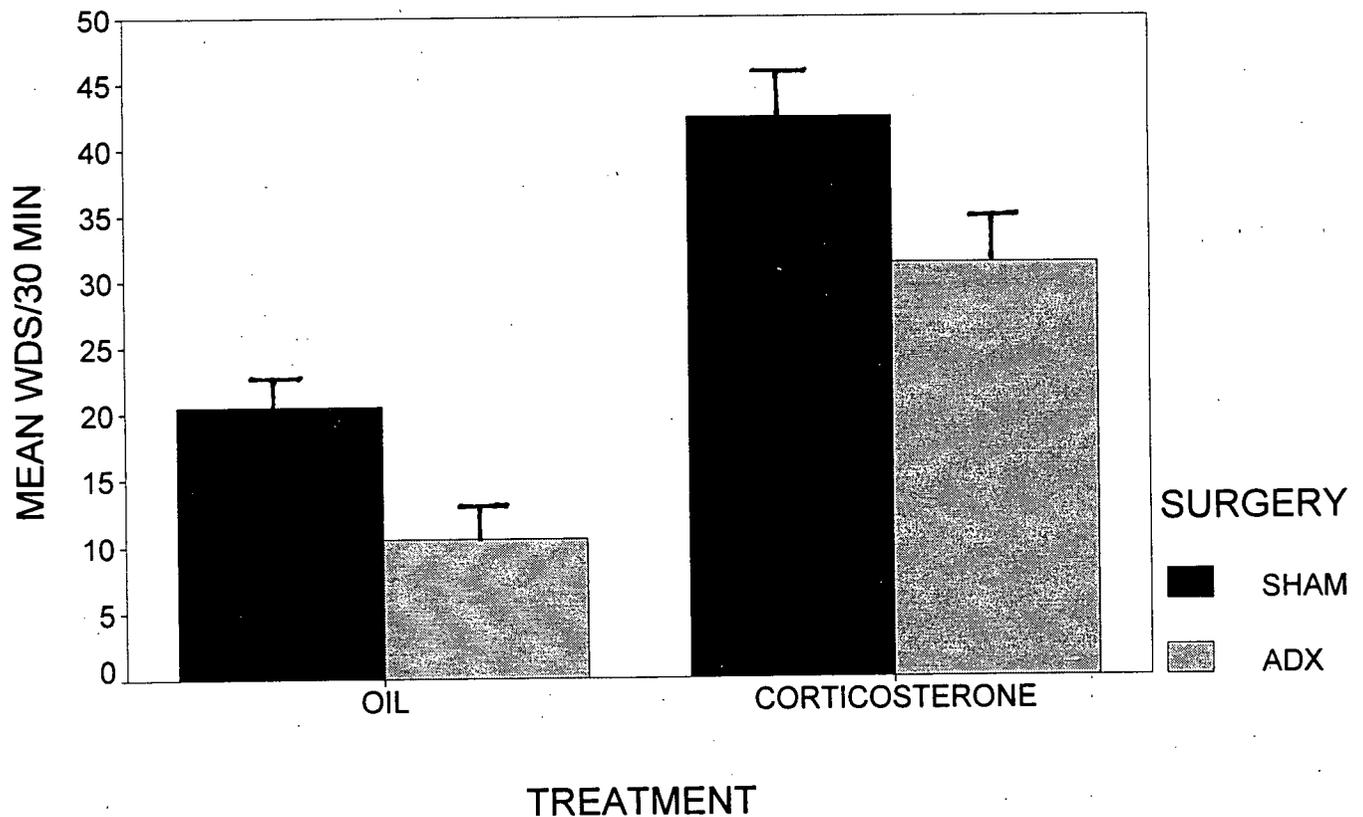


Figure 2: Effects of Chronic Corticosterone on DOI-Induced WDS in the Sham-Adrenalectomized (SHAM) and Adrenalectomized (ADX) Female Rat.

A comparison of the data obtained from both Experiments 2A and 2B using three-way ANOVA (surgery x treatment x gender) revealed no significant effect of gender on spontaneous WDS ($F(1,48) = 0.55, p = 0.46$) but showed that females displayed significantly more DOI-induced WDS ($F(1,48) = 60.45, p = 0.0001$) than the males.

The results of Experiment 2 replicate those of Experiment 1 in that adrenalectomy significantly inhibits WDS behavior. The present results also demonstrate that chronic corticosterone treatment reverses this effect. Additionally, corticosterone has the effect of significantly increasing the display of WDS in intact animals. Chronic corticosterone treatment has been shown to increase 5-HT_{2A} density in the neocortex (Kuroda et al., 1992), however, as noted earlier, WDS is believed to originate in either the midbrain or brainstem. It remains possible that chronic corticosterone treatment affects 5-HT_{2A} density in the regions of the brain responsible for the display of WDS.

EXPERIMENT 3

In Experiments 1 and 2 it was shown that the adrenal steroid corticosterone may be important in the regulation of 5-HT_{2A} receptor activity. A chronic elevation in corticosterone levels appears to increase 5-HT_{2A} activity as seen by an increase in the frequency of WDS. Since both elevated 5-HT_{2A} activity and WDS frequency have been related to a decrease in male sexual proficiency (Watson & Gorzalka, 1990), this leads to the hypothesis that chronic corticosterone treatment would result in an inhibition of male sexual behavior. Experiment 3 was designed to test this hypothesis.

Animals

Male and female Long Evans rats were obtained from Charles River Canada Inc., Montreal, at 60 days of age. The rats were housed separately by sex, in standard wire mesh cages. Thirty male rats of known copulatory experience were housed three per cage, and were approximately 140 days old when tested. Eighteen stimulus females were bilaterally ovariectomized while under a combination of sodium pentobarbital (50 mg/kg IP) and ketamine (0.5 mg/kg IP) anesthesia. Females were housed in groups of four, and were approximately 7 months old when testing commenced. All animals had free access to food and water and were maintained on a reverse 12/12 hr light cycle.

Experiment 3A Procedure

Fifteen male rats were randomly chosen to receive chronic corticosterone (CORT) and the remaining fifteen to receive oil chronically (OIL). Corticosterone-21-acetate was suspended in sesame oil (50 mg/ml) and was injected subcutaneously daily for 13 days (1 ml/kg). Control animals received sesame oil (1 ml/kg) for the same duration. Both spontaneous sexual behavior and sexual behavior following the administration of DOI were observed. On the 10th day all testing for spontaneous WDS and sexual behavior was conducted. Behavioral testing took place during the middle third of the dark cycle prior to that day's corticosterone/oil injection. Thirty minutes before being tested for spontaneous behavior, all rats were injected with 0.9% saline solution (1 ml/kg) subcutaneously. On the 14th day, rats were scored for behavior following the administration of DOI. DOI was dissolved in 0.9% saline solution (1 mg/cc) and a dose of 1 mg/kg was injected subcutaneously 30 minutes prior to behavioral testing. DOI-induced behavior was scored in the same manner as spontaneous behavior.

Behavioral Testing

Sexual receptivity was induced in Long Evans female rats by the subcutaneous injection of 10 µg estradiol benzoate (Steraloids, Wilton, NH) and 500 µg progesterone (Steraloids), 48 hrs and 4 hrs prior to testing, respectively. Both steroids were dissolved in 0.1 ml peanut oil. Males were placed in separate Plexiglas chambers and allowed to habituate for five minutes. Five males were monitored simultaneously by a trained observer who remained blind to the treatment conditions throughout the experiment. A receptive female was then placed with each male and the following sexual behavior parameters were scored: frequency of mounts with pelvic thrusting, frequency of intromissions, frequency of ejaculations, mount latency (i.e., the period between the introduction of the receptive female to the first mount by the male), intromission latency (i.e., the period between the introduction of the receptive female to the first intromission), ejaculation latency (i.e., the period between the first intromission and the first ejaculation) and the post-ejaculatory interval (i.e., the period between ejaculation and the first intromission of the next copulatory bout). Additionally, the frequency of WDS was recorded. Each testing period was 30 minutes, and stimulus females were rotated between males every ten minutes. This procedure was repeated for all of the groups.

Results were analyzed using independent samples t-tests. Levene's test for equality of variance was performed on each variable prior to performing t-test analyses. A Bonferonni adjustment of alpha was performed in order to decrease the probability of obtaining Type I errors. So that not all statistical power was lost due to Bonferroni adjustment, a familywise error rate of 0.10 was accepted. Therefore, the significance level was set to 0.0125 for each individual t-test performed on the above-mentioned measures of sexual behavior and WDS. Rats that failed to achieve ejaculation during the test interval were dropped from the data analyses of mounts and intromissions, and missing latency scores were set to the maximum (1800 s)

Experiment 3A Results*Spontaneous Condition:*

TABLE 5
EFFECTS OF CHRONIC CORTICOSTERONE (50 MG/KG) ON SPONTANEOUS WDS
AND SEXUAL BEHAVIOR (MEAN \pm SEM) IN THE MALE RAT

BEHAVIOR	OIL	CORT
SEXUAL BEHAVIORS		
mounts	11.67 \pm 1.15	11.38 \pm 1.34
intromissions	9.40 \pm 0.52	11.08 \pm 0.53
ejaculations	1.87 \pm 0.17	1.07 \pm 0.18
mount latency	113.33 \pm 23.00	136.40 \pm 30.12
intromission latency	259.33 \pm 39.90	368.93 \pm 54.67
ejaculation latency	623.20 \pm 75.97	919.00 \pm 117.43
post-ejaculatory interval	400.40 \pm 18.01	430.00 \pm 21.35
WDS BEHAVIOR	1.20 \pm 0.26	2.53 \pm 0.39

Data for spontaneous WDS and sexual behavior are presented in Table 5. Analyses revealed that chronic administration of corticosterone significantly decreased the number of ejaculations ($t(28) = 3.26$, $p = 0.003$), and increased the frequency of WDS ($t(28) = 2.84$, $p = 0.008$).

Although failing to reach statistical significance, ejaculation latency was notably longer ($t(28) =$

2.11, $p = 0.04$), and the number of intromissions prior to ejaculation was increased ($t(26) = 2.23$, $p = 0.04$) in animals receiving corticosterone. Mount latency ($t(28) = 0.61$, $p = 0.55$), number of mounts prior to ejaculation ($t(26) = 0.16$, $p = 0.87$), intromission latency ($t(28) = 1.62$, $p = 0.12$), and the post-ejaculatory interval ($t(26) = 1.07$, $p = 0.30$) were not significantly affected by chronic corticosterone treatment.

DOI Condition:

Data for DOI-induced WDS and sexual behavior are presented in Table 6. Similar trends were observed following the administration of DOI. While DOI had the effect of inhibiting sexual behavior in both groups, the inhibition was more pronounced in rats receiving corticosterone. Specifically, chronic corticosterone administration resulted in a significantly lengthened ejaculation latency ($t(14) = 3.03$, $p = 0.009$) and intromission latency ($t(28) = 2.91$, $p = 0.007$), and a decrease in the total number of ejaculations ($t(14) = 3.06$, $p = 0.009$). Because no rats in the group receiving corticosterone ejaculated, analyses on the number of mounts and intromissions prior to ejaculation could not be performed. Instead, analyses were run on the total number of mounts and intromissions observed during the test period. Analyses revealed that corticosterone administration had the effect of significantly reducing the total number of intromissions ($t(20.65) = 3.93$, $p = 0.001$) but had no effect on the total number of mounts ($t(24.72) = 0.74$, $p = 0.47$). Additionally, mount latency was not affected by corticosterone treatment ($t(28) = 0.02$, $p = 0.98$). Because no animals intromitted following their first ejaculation, analysis of the post-ejaculatory interval could not be performed. Furthermore, as hypothesized, chronic corticosterone administration significantly increased the display of WDS behavior ($t(20.98) = 3.69$, $p = 0.001$).

TABLE 6
EFFECTS OF CHRONIC CORTICOSTERONE (50 MG/KG) ON DOI-INDUCED WDS
AND SEXUAL BEHAVIOR (MEAN±SEM) IN THE MALE RAT

BEHAVIOR	OIL	CORT
SEXUAL BEHAVIORS		
mounts (total)	10.53 ± 2.01	8.73 ± 1.37
intromissions (total)	13.20 ± 2.09	4.00 ± 1.05
ejaculations	0.40 ± 0.13	0.00 ± 0.00
mount latency	406.93 ± 152.12	401.80 ± 158.50
intromission latency	607.67 ± 133.45	1161.13 ± 135.67
ejaculation latency	1353.60 ± 147.29	1800.00 ± 0.00
post-ejaculatory interval	-----	-----
WDS BEHAVIOR	14.80 ± 1.54	27.53 ± 3.06

Experiment 3B Procedure

Thirty days after the completion of Experiment 3A, rats were reassigned in a counterbalanced fashion to receive either corticosterone or oil chronically. The procedure, behavioral testing, and statistical analyses were identical to those employed in Experiment 3A with the exception that the dose of corticosterone was lowered to 20 mg/kg. As mentioned previously, both 50 mg/kg and 20 mg/kg doses of corticosterone, when administered chronically, result in an upregulation of 5-HT_{2A} receptor density in the neocortex of the rat (Kuroda et al., 1992).

Therefore, results from Experiment 3B (20 mg/kg) are expected to follow a similar trend as seen in Experiment 3A (50 mg/kg).

Experiment 3B Results

Spontaneous Condition:

Data for spontaneous WDS and sexual behavior are presented in Table 7. Similar results were obtained when the dose of corticosterone was lowered to 20 mg/kg. Corticosterone significantly inhibited ejaculatory behavior ($t(28) = 3.01, p = 0.005$) and increased WDS ($t(28) = 2.82, p = 0.009$). Although failing to reach statistical significance, there was a trend toward more intromissions being required in order to achieve ejaculation in the animals receiving corticosterone ($t(21) = 2.28, p = 0.03$). The number of mounts prior to ejaculation ($t(21) = 0.52, p = 0.61$), mount latency ($t(28) = 0.62, p = 0.54$), intromission latency ($t(28) = 0.54, p = 0.59$), ejaculation latency ($t(22.97) = 1.89, p = 0.07$), and post-ejaculatory interval ($t(21) = 1.14, p = 0.27$) did not differ significantly between groups.

TABLE 7
EFFECTS OF CHRONIC CORTICOSTERONE (20 MG/KG) ON SPONTANEOUS WDS
AND SEXUAL BEHAVIOR (MEAN \pm SEM) IN THE MALE RAT

BEHAVIOR	OIL	CORT
SEXUAL BEHAVIORS		
mounts	11.93 \pm 2.53	14.00 \pm 3.00
intromissions	10.00 \pm 0.55	12.10 \pm 0.79
ejaculations	1.80 \pm 0.20	0.87 \pm 0.24
mount latency	153.80 \pm 41.83	119.40 \pm 36.62
intromission latency	325.87 \pm 116.45	254.80 \pm 60.60
ejaculation latency	580.80 \pm 110.63	985.47 \pm 183.69
post-ejaculatory interval	360.92 \pm 23.50	402.33 \pm 26.63
WDS BEHAVIOR	1.53 \pm 0.35	3.13 \pm 0.45

DOI Condition:

Data for DOI-induced WDS and sexual behavior are presented in Table 8. Following the administration of DOI, corticosterone had the effect of lengthening the intromission latency ($t(28) = 2.95$, $p = 0.006$) and increasing WDS behavior ($t(28) = 2.86$, $p = 0.008$). Due to the low number of rats achieving ejaculation (OIL $n=5$, CORT $n=1$), analyses on the number of mounts and intromissions prior to ejaculation could not be performed. Therefore, as in the DOI condition of Experiment 3A, analyses were run on the total number of mounts and intromissions

observed during the test period. The total number of intromissions was reduced in the animals receiving corticosterone ($t(21.82) = 3.17, p = 0.004$), but the total number of mounts was unaffected ($t(28) = 0.42, p = 0.68$). Additionally, mount latency ($t(28) = 0.82, p = 0.42$) and number of ejaculations ($t(21.27) = 1.87, p = 0.08$) were not affected by corticosterone treatment. There was a trend towards lengthened ejaculation latency in the animals receiving corticosterone, however this measure failed to reach statistical significance ($t(17.16) = 2.18, p = 0.04$). Analysis of the post-ejaculatory interval could not be performed because animals failed to intromit following their first ejaculation.

TABLE 8
EFFECTS OF CHRONIC CORTICOSTERONE (20 MG/KG) ON DOI-INDUCED WDS
AND SEXUAL BEHAVIOR (MEAN \pm SEM) IN THE MALE RAT

BEHAVIOR	OIL	CORT
SEXUAL BEHAVIORS		
mounts (total)	13.53 \pm 2.49	12.07 \pm 2.27
intromissions (total)	9.33 \pm 1.84	2.67 \pm 1.02
ejaculations	0.33 \pm 0.12	0.07 \pm 0.07
mount latency	555.13 \pm 140.11	731.07 \pm 164.05
intromission latency	921.93 \pm 149.23	1463.80 \pm 107.24
ejaculation latency	1337.27 \pm 175.00	1740.80 \pm 59.20
post-ejaculatory interval	-----	-----
WDS BEHAVIOR	12.53 \pm 2.14	21.67 \pm 2.38

Discussion

The results of Experiment 3 indicate that chronic corticosterone administration inhibits sexual performance in the male rat as seen by an increase in ejaculation and intromission latencies, and a decrease in the total number of ejaculations and intromissions. These results are consistent with the finding that chronic stress inhibits sexual behavior in the male rat (D'Aquila et al., 1994). Sexual motivation did not appear to be affected since rats receiving corticosterone treatment mounted with the same frequency and latency as did the control animals. Sexual behavior was significantly inhibited at both doses of corticosterone and across both the spontaneous and DOI conditions. Additionally, corticosterone had the effect of increasing WDS across all conditions. In contrast to the results of Experiment 2, chronic corticosterone treatment significantly increased spontaneous WDS in Experiment 3. While the majority of rats did not display any spontaneous WDS behavior in Experiment 2, the majority of rats in Experiment 3 displayed at least one spontaneous WDS. While these results may suggest a strain difference in WDS behavior, it is more likely that the introduction of receptive females in Experiment 3 was responsible for increasing WDS behavior across both groups. It has previously been shown that the incidence of spontaneous WDS changes when rats of varying copulatory proficiency are in the presence of a receptive female (Watson & Gorzalka, 1990).

It has been suggested that WDS and copulatory behavior rely on overlapping neural mechanisms. Brainstem administration of DOI, in the region of the raphe obscurus, resulted in a dose-dependent decrease in sexual behavior and a concurrent increase in WDS (Watson & Gorzalka, 1992). The fact that neurons in the raphe nuclei have bifurcating axons that project to the medial preoptic area (MPOA) and the ventral horns of the cervical spinal cord (Leanza, Pellitteri, Russo & Stanzani, 1991) is of particular interest since the MPOA is a brain region

important for the display of male copulatory behavior (Rose, 1990) and the cervical spinal cord contains motoneurons which control the display of WDS. These results suggest the possibility that corticosterone may affect WDS and male sexual behavior by altering 5-HT_{2A} receptor density in the raphe nuclei. No radioligand binding studies have yet looked at the effect of corticosterone administration on 5-HT_{2A} receptor density in this region of the brain.

The observed increase in WDS in males receiving corticosterone supports previous findings that both stress (McKittrick et al., 1995) and chronic corticosterone treatment (Kuroda et al., 1992) increase 5-HT_{2A} activity. In addition, the previously noted evidence that decreases in male rat sexual behavior can be induced by increasing 5-HT_{2A} activity (Gorzalka et al., 1990; Watson & Gorzalka, 1991) support the current observations of corticosterone decreasing sexual proficiency. Overall, these results demonstrate that chronic corticosterone decreases sexual behavior and increases WDS in the male rat, and these effects are likely mediated by increased 5-HT_{2A} activity.

EXPERIMENT 4

In Experiment 3 it was shown that the adrenal steroid corticosterone inhibits male sexual behavior. The concurrent increase in WDS suggests that the effects of corticosterone on male sexual behavior may be mediated by an upregulation in 5-HT_{2A} activity. As noted earlier, 5-HT_{2A} activity mediates differential effects on male and female sexual behavior (Gorzalka, et al, 1990). In contrast to an inhibition of male sexual behavior, elevated 5-HT_{2A} activity has been related to a facilitation of female sexual behavior. This suggests that chronic corticosterone treatment would facilitate female sexual behavior via an upregulation in 5-HT_{2A} density. Experiment 4 investigated the effects of both chronic corticosterone and adrenalectomy on female sexual behavior and WDS.

Animals

This experiment used the same 16 ADX and 16 SHAM female rats as were employed in Experiments 1B and 2B. Females were approximately 5 months of age at the time of testing. Twenty sexually proficient Long-Evans males were used for eliciting sexual behavior. Males were housed in groups of four, and were approximately 8 months old when testing commenced. All animals had free access to food and water and were maintained on a reverse 12/12 hr light cycle.

Experiment 4A Procedure

Half of the adrenalectomized rats were randomly chosen to receive corticosterone chronically (ADX-CORT n=8), while the other half received propylene glycol chronically (ADX-OIL n=8). The sham-adrenalectomized rats were divided similarly (SHAM-CORT n=8; SHAM-OIL n=8). Corticosterone-21-acetate was suspended in propylene glycol (20 mg/ml) and was injected subcutaneously daily for 13 days (1 ml/kg). Control animals received propylene glycol (1 ml/kg) for the same duration. On the 10th day, all females were tested twice for spontaneous behavior. Behavioral testing took place during the middle third of the dark cycle prior to that day's corticosterone/propylene glycol injection. One week, forty-eight hours, and twenty-four hours prior to the time of the first spontaneous test all females were injected with estradiol benzoate (10 µg dissolved in 0.1 ml peanut oil, SC). Thirty minutes before the first spontaneous behavior test, all rats were injected with 0.9% saline solution (1 ml/kg) subcutaneously. Upon completion of the first spontaneous test all females were injected with progesterone (50 µg dissolved in 0.1 ml peanut oil, SC). The second spontaneous test occurred approximately 4 hours after the administration of the progesterone. Again, thirty minutes before behavioral testing, all rats were subcutaneously injected with 0.9% saline solution (1 ml/kg). Behavior for estrogen- and progesterone-primed females was scored in the same manner as for females primed with

estrogen alone.

Behavioral Testing

Behavioral testing began approximately 8 weeks following adrenalectomy. Each rat was exposed to a total of four test sessions. The test session began with the presentation of the female to a sexually experienced male in individual test chambers. Proceptive, receptive, rejection and WDS behaviors were recorded simultaneously by a trained observer who remained blind to the treatment of the rats. Sexual receptivity was assessed by scoring lordosis behavior and calculating the lordosis quotient (LQ = the proportion of lordoses exhibited by a female in response to 10 mounts with pelvic thrusting by a male). If a male did not mount, the female was placed with a different male in another test chamber. Proceptive behaviors recorded included darts and ear wiggles. Females were also rated for the amount of sexual rejection (i.e., kicking, boxing, vocalizations) displayed on a scale of 0 (no rejection) to 3 (complete rejection). The test session was finished after the female had been mounted 10 times. The length of each test session was timed and a score of darts, ear wiggles, and WDS per minute was calculated. Data for both proceptive behaviors, darts and ear wiggles, were combined to form a composite proceptivity score of solicitations per minute.

Results were analyzed using two-way ANOVA and pairwise comparisons using the Newman-Keuls test. A Bonferroni adjustment of alpha was performed in order to decrease the probability of obtaining Type I errors. So that not all statistical power was lost due to Bonferroni adjustment, a familywise error rate of 0.10 was accepted. Therefore, the significance level was set to 0.025 for each individual ANOVA performed on the above-mentioned measures of sexual behavior and WDS.

Experiment 4A Results

Estrogen:

All data for spontaneous WDS and sexual behavior in estrogen-primed females are given in Table 9.

Receptivity was significantly affected by both corticosterone administration ($F(1,28) = 15.78$, $p = 0.001$) and adrenalectomy ($F(1,28) = 7.67$, $p = 0.01$). Corticosterone had the effect of facilitating lordosis regardless of the surgical condition. ADX animals displayed significantly more lordosis behavior than the SHAM animals in both the OIL and CORT treatment groups. The ADX-OIL and the SHAM-CORT did not differ significantly from each other.

Proceptive behavior was significantly affected by both corticosterone ($F(1,28) = 13.60$, $p = 0.001$) and adrenalectomy ($F(1,28) = 11.82$, $p = 0.002$). Similar to its effects on receptivity, corticosterone had the effect of increasing proceptivity regardless of the surgical condition of the animal. While adrenalectomy was found to facilitate receptivity, it significantly inhibited the display of proceptivity across treatment groups. Pairwise comparisons revealed that all groups differed significantly from each other with the exception of the SHAM-OIL and ADX-CORT groups.

TABLE 9
EFFECTS OF CHRONIC CORTICOSTERONE (20 MG/KG) AND ADRENALECTOMY ON
SPONTANEOUS WDS AND SEXUAL BEHAVIOR (MEAN±SEM)
IN THE ESTROGEN-PRIMED FEMALE RAT

GROUP	LQ	Solicitations per min	Rejection	WDS per min
SHAM-OIL	42.50 ± 4.53	0.61 ± 0.15	1.13 ± 0.40	0.09 ± 0.05
ADX-OIL	56.25 ± 4.98	0.24 ± 0.08	2.25 ± 0.16	0.10 ± 0.06
SHAM-CORT	62.50 ± 5.90	1.44 ± 0.26	0.50 ± 0.19	0.15 ± 0.08
ADX-CORT	77.50 ± 5.26	0.65 ± 0.13	1.38 ± 0.26	0.09 ± 0.04

Rejection behavior was significantly decreased by corticosterone treatment ($F(1,28) = 7.75$, $p = 0.01$) and increased by adrenalectomy ($F(1,28) = 13.79$, $p = 0.001$). All possible pairwise comparisons revealed statistical significance except that there was no difference between SHAM-OIL and ADX-CORT groups.

WDS was not affected by either corticosterone ($F(1,28) = 0.22$, $p = 0.65$) or adrenalectomy ($F(1,28) = 0.14$, $p = 0.71$).

No significant interactions were revealed for any of the above-mentioned behaviors.

Results indicate that chronic corticosterone has the effect of facilitating female sexual behavior as seen by an increase in both receptivity and proceptivity and a decrease in rejection behavior. It remains uncertain whether the facilitatory effects of corticosterone on female sexual

behavior are mediated by 5-HT_{2A} activity since corticosterone failed to influence WDS. It was previously observed in Experiment 2 that the effects of corticosterone on WDS only become apparent after the administration of DOI, possibly due to the low frequency with which spontaneous WDS occurs.

Adrenalectomy was found to have mixed effects on female sexual behavior. Receptive responding was increased indicating a facilitation of female sexual behavior, however, the frequency of proceptive behavior decreased and the display of rejection increased, suggesting an inhibitory effect of adrenalectomy on female sexual behavior.

Estrogen and Progesterone:

All data for spontaneous WDS and sexual behavior in estrogen- and progesterone-primed females are presented in Table 10.

After the administration of progesterone, results for receptivity were similar to results obtained when females were primed with only estrogen. Both corticosterone administration ($F(1,28) = 13.68, p = 0.001$) and adrenalectomy ($F(1,28) = 7.86, p = 0.01$) facilitated lordosis behavior. All groups differed significantly from each other except for the ADX-OIL and SHAM-CORT groups.

Corticosterone administration significantly increased the number of solicitations per minute ($F(1,28) = 25.59, p = 0.0001$). Adrenalectomy had no effect on proceptive behavior ($F(1,28) = 0.68, p = 0.42$). Pairwise comparisons revealed that both SHAM-OIL and ADX-OIL groups differed significantly from both SHAM-CORT and ADX-CORT groups.

TABLE 10
EFFECTS OF CHRONIC CORTICOSTERONE (20 MG/KG) AND ADRENALECTOMY ON
SPONTANEOUS WDS AND SEXUAL BEHAVIOR (MEAN \pm SEM)
IN THE ESTROGEN- AND PROGESTERONE-PRIMED FEMALE RAT

GROUP	LQ	Solicitations per min	Rejection	WDS per min
SHAM-OIL	58.75 \pm 5.49	3.37 \pm 0.25	0.25 \pm 0.16	0.11 \pm 0.05
ADX-OIL	76.25 \pm 6.25	3.28 \pm 0.30	0.38 \pm 0.26	0.16 \pm 0.06
SHAM-CORT	81.25 \pm 6.93	6.47 \pm 0.74	0.00 \pm 0.00	0.60 \pm 0.12
ADX-CORT	95.00 \pm 2.67	5.68 \pm 0.69	0.13 \pm 0.13	0.64 \pm 0.13

Neither corticosterone treatment ($F(1,28) = 2.24, p = 0.15$) nor adrenalectomy ($F(1,28) = 0.56, p = 0.46$) had any significant effect on rejection behavior.

In the presence of progesterone the facilitatory effect of corticosterone on WDS became apparent ($F(1,28) = 26.61, p = 0.0001$) but no effect of adrenalectomy on WDS was seen ($F(1,28) = 0.23, p = 0.63$).

Analyses revealed no significant interaction of corticosterone treatment and surgical condition on any measure of sexual behavior or WDS.

Interestingly, the administration of progesterone appears to have increased the overall frequency of WDS. A paired-samples t-test performed on the WDS displayed by females receiving estrogen alone and by females receiving estrogen in combination with progesterone

confirmed this ($t(31) = 4.02, p = 0.001$).

Following the administration of progesterone, corticosterone continued to increase both receptive and proceptive behavior while also increasing the display of WDS. These results provide evidence that the facilitatory effects of corticosterone on female sexual behavior are mediated by 5-HT_{2A} activity. While adrenalectomy facilitated lordosis even in the presence of progesterone, the administration of progesterone prevented the inhibition of proceptivity and the increase of rejection behavior.

Experiment 4B Procedure

When both spontaneous tests were completed, the females were returned to their home cages and the corticosterone and propylene glycol treatments were continued up to and including Day 13. On the 14th day all females were scored twice for WDS and sexual behavior following the administration of DOI. Forty-eight hours, and twenty-four hours prior to the time of the first DOI test, all females were injected with estradiol benzoate (10 µg dissolved in 0.1 ml peanut oil, SC). DOI was dissolved in 0.9% saline solution (1 mg/ml) and a dose of 1 mg/kg was injected subcutaneously 30 minutes prior to both behavioral test sessions. Upon completion of the first DOI test all females were injected with progesterone (50 µg dissolved in 0.1 ml peanut oil, SC). The second DOI test occurred approximately 4 hours after the administration of progesterone. Thirty minutes prior to the second DOI behavioral test, all rats were injected subcutaneously with 1 mg/kg DOI. DOI-induced behavior was scored in the same manner as spontaneous behavior. Previous studies in our lab have shown that DOI (1 mg/kg, SC) exerts behavioral effects 30 minutes to 3 hours after administration. In the present study, the second DOI injection was given approximately 4 hours after the first DOI injection. Therefore, the first DOI injection should no longer be exerting any significant effects on WDS or sexual behavior at the time of the second DOI behavioral test.

Experiment 4B Results

Estrogen:

All data for DOI-induced WDS and sexual behavior in estrogen-primed females are presented in Table 11.

TABLE 11
EFFECTS OF CHRONIC CORTICOSTERONE (20 MG/KG) AND ADRENALECTOMY ON
DOI-INDUCED WDS AND SEXUAL BEHAVIOR (MEAN±SEM)
IN THE ESTROGEN-PRIMED FEMALE RAT

GROUP	LQ	Solicitations per min	Rejection	WDS per min
SHAM-OIL	68.75 ± 5.15	2.62 ± 0.21	0.13 ± 0.13	0.77 ± 0.11
ADX-OIL	87.50 ± 3.13	1.16 ± 0.21	0.25 ± 0.16	0.32 ± 0.04
SHAM-CORT	90.00 ± 4.23	6.25 ± 0.79	0.25 ± 0.16	1.48 ± 0.15
ADX-CORT	93.75 ± 3.75	4.92 ± 0.57	0.25 ± 0.16	1.31 ± 0.15

Following the administration of DOI, both corticosterone administration ($F(1,28) = 11.07, p = 0.002$) and adrenalectomy ($F(1,28) = 7.41, p = 0.01$) facilitated lordosis behavior. Pairwise comparisons revealed that the SHAM-OIL group displayed significantly less lordosis than the other three groups which did not differ significantly from each other.

Proceptive behavior was significantly affected by both corticosterone treatment ($F(1,28) = 52.53, p = 0.0001$) and adrenalectomy ($F(1,28) = 7.52, p = 0.01$). While corticosterone treatment increased the number of solicitations per minute, adrenalectomy decreased the display of proceptive behavior. Pairwise comparisons revealed that all groups differed significantly from each other.

Neither corticosterone treatment ($F(1,28) = 0.16, p = 0.69$) nor adrenalectomy ($F(1,28) = 0.16, p = 0.69$) had any significant effect on rejection behavior.

Adrenalectomy reduced DOI-induced WDS ($F(1,28) = 6.53, p = 0.016$) and corticosterone treatment increased the display of WDS ($F(1,28) = 48.78, p = 0.0001$). Pairwise comparisons showed that all groups differed significantly except for SHAM-CORT and ADX-CORT.

No significant interactions of corticosterone treatment and adrenalectomy were found for WDS or for any measure of sexual behavior.

Following the administration of DOI, corticosterone facilitated both receptive and proceptive behavior, and increased WDS. Adrenalectomy increased lordosis behavior and decreased WDS but had no effect on proceptive behavior. These results are consistent with the hypothesis that the facilitation of female sexual behavior by corticosterone is mediated by an upregulation of 5-HT_{2A} density.

Estrogen and Progesterone:

All data for DOI-induced WDS and sexual behavior in estrogen- and progesterone-primed females are presented in Table 12.

Neither corticosterone treatment ($F(1,28) = 0.03$, $p = 0.87$) nor adrenalectomy ($F(1,28) = 0.25$, $p = 0.62$) had any significant effects on lordosis behavior. The lack of statistical significance for receptivity likely reflects a ceiling effect. Theoretically, the maximal lordosis quotient is 100%. In the present study, the control group (SHAM-OIL) had a mean LQ of 95%.

Corticosterone treatment increased the number of solicitations per minute ($F(1,28) = 19.89$, $p = 0.001$). Adrenalectomy had no effect on proceptive behavior ($F(1,28) = 0.19$, $p = 0.67$). Both SHAM-OIL and ADX-OIL groups differed significantly from both SHAM-CORT and ADX-CORT groups.

No females displayed any rejection behavior, so analyses on rejection were not performed.

WDS was significantly facilitated by corticosterone administration ($F(11,28) = 37.46$, $p = 0.0001$) and inhibited by adrenalectomy ($F(1,28) = 6.02$, $p = 0.02$). Pairwise comparisons revealed that all groups differed significantly from each other.

No significant interactions between corticosterone treatment and adrenalectomy were found for WDS or for any measure of sexual behavior.

Unlike the results for Experiment 4A, the administration of progesterone did not alter the overall frequency of DOI-induced WDS as revealed by a paired-samples t-test ($t(31) = 1.58$, $p = 0.12$).

Following the administration of DOI, chronic corticosterone treatment increased both proceptive behavior and WDS in estrogen and progesterone-primed females. The only significant effect of adrenalectomy was to inhibit WDS.

TABLE 12
EFFECTS OF CHRONIC CORTICOSTERONE (20 MG/KG) AND ADRENALECTOMY ON
DOI-INDUCED WDS AND SEXUAL BEHAVIOR (MEAN \pm SEM)
IN THE ESTROGEN- AND PROGESTERONE-PRIMED FEMALE RAT

GROUP	LQ	Solicitations per min	Rejection	WDS per min
SHAM-OIL	95.00 \pm 2.67	8.20 \pm 0.73	0.00 \pm 0.00	0.66 \pm 0.09
ADX-OIL	93.75 \pm 3.75	8.50 \pm 0.45	0.00 \pm 0.00	0.30 \pm 0.12
SHAM-CORT	91.25 \pm 5.15	12.79 \pm 0.92	0.00 \pm 0.00	1.29 \pm 0.13
ADX-CORT	96.25 \pm 2.63	11.73 \pm 1.23	0.00 \pm 0.00	1.10 \pm 0.12

Discussion

Taken together, results from Experiment 4 show that chronic corticosterone administration facilitates both sexual receptivity and proceptivity and increases WDS. These results conflict with reports that chronic corticosterone inhibits lordosis behavior (deCatanzaro & Gorzalka, 1980; deCatanzaro et al., 1981). The doses of corticosterone used in the studies which have found an inhibition of receptivity (ranging from 5 μ g/kg to 4mg/kg) were significantly lower than the dose used in the current study (20 mg/kg). The dose used in the current study was the same dose that has been reported to significantly increase 5-HT_{2A} density (Kuroda et al., 1992). It is possible that lower doses of chronically administered corticosterone do not alter 5-HT_{2A} density and act to inhibit lordosis through a different mechanism of action. This is the first study to look at the effects of chronic corticosterone on sexual proceptivity and

rejection.

A recent study has shown that chronic psychosocial stress increased both sexual receptivity and proceptivity in estrogen-primed females (Williams, et al, 1992). The psychosocial stressors used in that study significantly elevated serum corticosterone levels. The authors suggested that adrenal corticosterone is responsible for the increase in stress-induced receptivity and proceptivity by competitively binding to progestin receptors. Both glucocorticoids and progestins have receptors that are similar in structure (Carlstedt-Duke, Stromstedt, Persson, Cederlund, Gustafsson & Jornvall, 1988) and the hormones have been shown to have comparable affinities for various protein receptors (Trueba, Guantes, Vallejo, Sancho, Marino & Macarulla, 1987). However, acute administration of glucocorticoids fails exert any effect on female sexual receptivity when given at doses at which progesterone is effective (Gorzalka & Whalen, 1977). These latter findings suggest that it is unlikely that the stress-induced facilitation of female sexual behavior is the result of glucocorticoids binding to progesterone receptors. Another possibility is that stress-induced increases in adrenal progestin secretion act to facilitate female sexual behavior. Ovariectomized, estrogen-primed female rats that were chronically stressed by exercise showed increased receptive behavior and also demonstrated weight loss (Schwartz, Nunez & Axelson, 1983). The authors of this study argued that stress-induced adrenal progestin was not responsible for the effect on sexual behavior since estrogen possesses weight reducing properties (Bernstein, Courtney & Braget, 1986) which are reversed by progesterone (Tchekmedyan, Hickman & Heber, 1991).

The significant increase in WDS evident in the present results suggests an alternative mechanism for the facilitating effects of stress on female sexual behavior. Adrenal corticosterone may be interacting with the 5-HT_{2A} system, since WDS are accepted to be primarily mediated by 5-HT_{2A} activity. Support for this has been demonstrated with an increase in radioactively labelled 5-HT_{2A} receptors and WDS after corticosterone

administration (Kuroda et al., 1992). These physiological and behavioral changes occurred without any changes in plasma 5-HT/5-HIAA levels, thus indicating that if corticosterone is involved in 5-HT_{2A} activity, it does so independently of changes in circulating 5-HT levels.

Additionally, adrenalectomy increases lordosis behavior and, in the absence of progesterone, inhibits proceptive behavior. Studies on the effects of adrenalectomy have yielded conflicting results, reporting either a facilitation of receptivity (Gorzalka & Raible, 1981; Gray & Gorzalka, 1980) or a failure to affect receptivity (Kow & Pfaff, 1975; Larsson et al., 1974). It has been suggested that adrenalectomy may facilitate receptivity by elevating levels of ACTH or by facilitating the action of monoamine oxidase, an enzyme which metabolizes both catecholamines and serotonin (deCatanzaro & Gorzalka, 1980; deCatanzaro et al., 1981). It has recently been demonstrated that the adrenal gland is important for the display of proceptive behavior (Gorzalka & Moe, 1994; Fenandez-Guasti, Vega-Matuszczyk & Larsson, 1991). Proceptive behavior is thought to be more dependent on progesterone than receptive behavior (Tennent, Smith & Davidson, 1980). Progesterone of adrenal origin may contribute to the display of proceptive behavior in estrogen-primed females. Adrenalectomy may inhibit proceptivity by removal of this endogenous source of progesterone.

The two consecutive injections of DOI given 4 hours apart in Experiment 4B may be problematic. Even though the behavioral effects related to the first DOI injection were no longer apparent at the time of the second DOI behavioral test, there is evidence to suggest that the acute administration of DOI exerts rapid effects on 5-HT_{2A} receptor density. It has been shown that 4-24 hours after acute administration of DOI, 5-HT_{2A} receptor density is significantly reduced (Buckholtz, Zhou & Freedman, 1988). Additionally, a single injection of DOI significantly reduced 5-HT_{2A}-mediated head twitches following a second injection of DOI 24 hours later (Darmani, Martin & Glennon, 1992). It is possible that the second DOI test may have been confounded by a decrease in 5-HT_{2A} receptor density due to the first DOI injection.

The administration of progesterone induced a significant increase in WDS in Experiment 4A but not Experiment 4B. The downregulation of 5-HT_{2A} receptors due to DOI administration may have obscured the facilitating effects of progesterone on WDS. There is no obvious explanation for the facilitation of WDS by progesterone in Experiment 4A. It is possible that progesterone may in some way modulate 5-HT_{2A} activity. Progesterone has been shown to increase the 5-HIAA/5-HT ratio in estrogen-primed receptive female rats (Wilson & Hunter, 1985). This suggests that progesterone facilitates female sexual behavior by increasing 5-HT activity on 5-HT_{2A} receptors. If this were the case then the adrenalectomy-induced reduction of WDS observed in Experiments 1 and 2 may also be linked to the absence of adrenal progesterone. Further investigation is required to determine the role of progesterone in the mediation of WDS.

GENERAL DISCUSSION

In Experiment 1 it was shown that adrenalectomy significantly reduced WDS behavior following the administration of the 5-HT_{2A} agonist DOI. Chronic corticosterone treatment was shown to effectively block the adrenalectomy-induced reduction of DOI-induced WDS and increase the frequency of DOI-induced WDS in intact animals in Experiment 2. In Experiment 3, the effects of chronic corticosterone on WDS and concurrent male sexual behavior were investigated. The corticosterone treatment resulted in an increase in WDS behavior and an inhibition of sexual behavior in both the spontaneous and DOI-induced conditions. In Experiment 4, the effects of both chronic corticosterone and adrenalectomy on WDS and concurrent female sexual behavior were investigated. Corticosterone increased WDS and facilitated female sexual behavior while adrenalectomy decreased WDS and had mixed effects on female sexual behavior.

One might speculate that the increased WDS seen in Experiments 1-4 reflects an increase in 5-HT_{2A} receptor affinity as opposed to an actual increase in receptor number or density.

However, radioligand binding studies have demonstrated that manipulations of the HPA axis alter the number of 5-HT_{2A} receptors as opposed to the affinity of the receptor (Kuroda et al., 1992; Martire et al., 1989). This suggests that the results of the present study reflect an upregulation in 5-HT_{2A} density.

The mechanism of 5-HT_{2A} upregulation by adrenalectomy and chronic corticosterone treatment is not understood. Lowered presynaptic serotonergic activity could be the cause of the increase in 5-HT_{2A} density, however, this is not likely. The regulation of 5-HT_{2A} receptors does not follow the classical rules of receptor regulation. Instead of upregulating after antagonist administration, 5-HT_{2A} receptors downregulate (Sanders-Bush, 1990). Also, the lowered levels of 5-HT by denervation, neurotoxins, and synthesis inhibitors does not modify 5-HT_{2A} receptor density (Eison & Mullins, 1996). Additionally, increased 5-HT_{2A} receptors are seen in adrenalectomized and chronically ACTH-treated rats without any modifications in the levels of 5-HT or its major metabolite, 5-hydroxyindole acetic acid (Kuroda et al., 1992). Therefore, it is possible that the upregulation of 5-HT_{2A} receptors occurs independently of presynaptic events.

The existence of two distinct glucocorticoid receptor (GR) systems in the rat brain has been well established. The type I GR displays a high affinity for corticosterone and is primarily located in the lateral septum and hippocampus. The type II GR displays a low affinity for corticosterone and a much higher affinity for synthetic glucocorticoids such as dexamethasone, and is widely distributed throughout the brain (Reul & deKloet, 1985). The type II GR only becomes fully occupied with corticosterone when endogenous levels of corticosterone attain stress levels (Dallman, Akana, Cascio, Darlington, Jacobsen & Levin, 1987). The corticosterone doses used in the present study are sufficiently high to occupy both GR systems (Reul & deKloet, 1985). Chronic treatment with dexamethasone has been found to significantly increase 5-HT_{2A} receptor density in the cerebral cortex but not in the hippocampus (Kuroda, Mikuni,

Nomura & Takahashi, 1993). These results suggest that the type II GR is involved in the regulation of 5-HT_{2A} receptor density. However, as mentioned previously, glucocorticoids and progestins may compete for the same receptor site (Trueba et al., 1987). Additionally, progesterone has been shown to alter 5-HT_{2A} receptor density in female rats (Biegon, Reches, Snyder & McEwen, 1983), and in the present study, alter the 5-HT_{2A} receptor mediated behavior WDS in estrogen-primed females. Therefore, it is possible that dexamethasone affects 5-HT_{2A} receptor density via interactions on the progesterone receptor. If the effects of dexamethasone on 5-HT_{2A} receptor density could be blocked by the administration of a selective type II GR antagonist this would provide further evidence that the type II GR modulates 5-HT_{2A} receptor density. Unfortunately, to date, no selective type II GR antagonist is available. All currently available type II GR antagonists, such as RU-28362 and RU-38486, also possess antiprogestin activities (Mao, Regelson & Kalimi, 1992). Further investigation is necessary to reveal the interaction between glucocorticoids and progestins in modulating 5-HT_{2A} receptor activity.

In Experiments 1 and 2, it was found that females displayed significantly more WDS than males. Since testosterone has been shown to interact with 5-HT_{2A} mediated behaviors (Gonzalez et al, 1993), and DOI influences male sexual behavior through a neural system that is modulated by testosterone (Padoin & Lucion, 1995) it is possible that testosterone acts in some manner to inhibit the display of WDS. It has been demonstrated that both chronic and acute estrogen act to increase 5-HT_{2A} receptor density (Biegon et al., 1983; Sumner & Fink, 1995), however, it is not likely that estrogen could have influenced WDS since the ovariectomized female rats in Experiments 1 and 2 did not receive estrogen. Another possibility for the apparent sex difference in WDS may be related to sex differences in HPA function. Sex differences in HPA function have been well established. Female rats show greater glucocorticoid responses to stress (Kant, Lenox, Bunnell, Mougey, Pennington & Meyerhoff, 1983) and have a greater number of central corticosterone binding sites (Turner & Weaver, 1985). In general, it appears

that testosterone acts to inhibit HPA function while estrogen enhances HPA function (Handa, Burgess, Kerr & O'Keefe, 1994). It is also possible that the sex difference in WDS observed in Experiment 1 relates to effects of ovariectomy. Ovariectomy increases GR mRNA in selective regions of the rat brain (Peiffer, Lapointe & Barden, 1991).

Acute administration of the 5-HT_{2A} agonist DOI has been shown to reduce the regional cerebral metabolic rate for glucose (rCMR_{glc}) in the hippocampus and thalamus but not in other brain regions (Freo, Holloway, Kalogeras, Rapoport & Soncrant, 1992). It has also been demonstrated that adrenalectomy abolishes the metabolic effects of DOI in the hippocampus, while enhancing DOI's metabolic effects in the thalamus. These results suggest that some of the brain responses to DOI are dependent on an intact HPA axis. Therefore, it is possible that the decrease in DOI-induced WDS observed in adrenalectomized animals reflects the inability of DOI to alter the metabolism in the regions of the brain responsible for the display of WDS. Additionally, this may explain why the effect of adrenalectomy on WDS only became apparent after the administration of DOI in Experiments 1, 2, and 4. Kuroda and colleagues (1992) reported that while adrenalectomy significantly reduced DOI-induced WDS, there was no alteration in 5-HT_{2A} receptor binding in the neocortex. Indeed, these authors speculated that the effects of adrenalectomy on WDS may be mediated by some non-specific effect due to the lack of adrenal steroids. However, as previously mentioned, the WDS is believed to originate somewhere in the brainstem and/or midbrain and not in the neocortex. Further investigation is necessary to reveal the mechanism by which adrenalectomy alters DOI-induced WDS.

Taken together, the results from the present series of experiments suggest that corticosterone's effect in facilitating and inhibiting female and male rat sexual behavior, respectively, and increasing WDS in both females and males, reflects increased 5-HT_{2A} activity. The present series of experiments has demonstrated that the adrenal hormone corticosterone may be important in the regulation of 5-HT_{2A}-mediated behaviors. The corticosterone

administered in this study was given at doses and over a period of time that have been demonstrated to result in an increase in 5-HT_{2A} binding in the neocortex of the rat (Kuroda et al., 1992). Combined, these results suggest that the effect of corticosterone on behavior may be mediated by an increase in 5-HT_{2A} receptor activity.

The present results have implications for the effects of stress on psychiatric disorders, such as depression, that have been related to an upregulation in 5-HT_{2A} receptor density. A primary physiological response to stress is activation of the HPA axis - in particular, during prolonged periods of stress, there is a chronic elevation in cortisol and corticosterone levels. Naturally-occurring chronic elevations in corticosterone have been shown to be proportionately related to increases in 5-HT_{2A} receptor binding in the parietal cortex (McKittrick et al., 1995). It has been suggested that these alterations in serotonergic binding during periods of stress are part of an adaptive response, but it has also been demonstrated that increased 5-HT_{2A} activity is anxiogenic and actually exacerbates negative behavioral effects during stress (Deakin, 1988). Indeed, stress has been shown to be one of the predisposing factors for depression (Gold, Goodwin & Chrousos, 1988), which, as mentioned previously, has been associated with an increase in 5-HT_{2A} activity. Several studies have reported that patients with depressive illness tend to show HPA hormonal changes consistent with the changes seen during times of stress: patients tend to have elevated basal cortisol levels (Murphy, 1991), higher levels of corticotropin-releasing hormone in the cerebrospinal fluid (Nemeroff, Widerlov & Bissette, 1984), and impaired cortisol suppression in response to dexamethasone (Arana & Mossman, 1988). The alterations in the serotonergic system which are induced by prolonged elevation of corticosterone are opposed by changes seen following antidepressant therapy (Heninger & Charney, 1987).

The 5-HT_{2A} receptor has received much attention in its involvement with depression. Numerous postmortem studies have reported increased 5-HT_{2A} receptors in the brains of

people suffering from depression which may be the result of low levels of 5-HT (Arora & Meltzer, 1989; Mann, Arango, Marzuk, Theccanat & Reis, 1989). Consistent with this is the observation that antidepressants function as 5-HT_{2A} antagonists and have the ability to decrease 5-HT_{2A} receptor density in humans (Deakin, 1988) and rats (Peroutka & Snyder, 1980a, 1980b), and may do so by increasing 5-HT release (Ogren, Fuxe & Agnati, 1985). However, it has been noted that 5-HT_{2A} receptors do not follow classical rules of receptor regulation. Therefore, the increase in 5-HT_{2A} receptors evident in the brains of depressed patients cannot be explained by a decrease in 5-HT neurotransmission. This makes findings in the depression literature difficult to interpret since there are both decreased 5-HT and increased 5-HT_{2A} receptors in depressed patients (Mann et al., 1989); however, it has been stated that decreased 5-HT does not induce increases in 5-HT_{2A} receptors. The regulation of 5-HT_{2A} receptors is paradoxical, and it involves mechanisms that are unlike those of traditional monoaminergic receptors (Eison & Mullins, 1996). The mechanism responsible for the increase in 5-HT_{2A} receptors in the brains of depressed patients is thought to involve the HPA axis.

Depression has been studied and described both in the context of HPA axis disruption and 5-HT_{2A} upregulation. It is possible that the 5-HT_{2A}-HPA axis interaction may be relevant to depression. It has been shown that the increase in 5-HT_{2A} receptor density in chronically stressed rats was proportional to the extent of HPA axis disruption (Martire et al., 1989). The interaction between 5-HT_{2A} receptors and the HPA axis was studied in rats given corticosterone (Kuroda et al., 1992) where it was suggested that the HPA axis regulated the increase in 5-HT_{2A} receptors. Depression, which has been modelled in the rat using a learned helplessness procedure, was shown to induce 5-HT_{2A} receptor hypersensitivity and increased WDS (Natelson, Ottenweller, Cook, Pitman, McCarty, & Tapp, 1988). It is possible that the increase in 5-HT_{2A}-mediated WDS and changes in sexual behavior seen in the subjects receiving chronic stress reflect an upregulation of 5-HT_{2A} receptor density. This response to adrenal corticosterone secretion and HPA axis disruption suggest that chronic stress may

predispose one to depression by increasing 5-HT_{2A} receptors. Results from the present study have implications for the pathophysiology and treatment of the depressive disorders by suggesting a neuroendocrine pathway through which both stress and depression are mediated

Additionally, the sex differences in HPA function and 5-HT_{2A} receptor mediation mentioned previously suggest a potential link between sex hormones and depression. Women have a higher incidence of depression (Weissman & Klerman, 1977) and depression is often associated with physiological states relating to ovarian hormone secretion such as postpartum depression and premenstrual tension (Hopkins, Marcus & Campbell, 1984; Abramowitz, Baker & Fleischer, 1982). Studies have demonstrated that the 5-HT_{2A} downregulating effect of antidepressants is dependent on the presence of moderate circulating levels of either estrogen, progesterone or testosterone (Kendall, Stancel & Enna, 1981, 1982). This suggests that the effectiveness of antidepressants may be dramatically affected with alterations in gonadal hormone secretion. Adjunctive hormonal therapy may be necessary for depressed patients that fail to respond to conventional antidepressant therapies. Further investigation is necessary to more clearly define the role of gonadal hormones in the modulation of 5-HT_{2A} receptor activity, HPA axis activity, and the etiology of depression.

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