The inhibitory influence of HPA axis hormones on the 5-HT$_{2A}$ receptor-mediated behavioural effects of risperidone: Potential therapeutic implications

JANIE J. HONG

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Department of \textit{Psychology}

The University of British Columbia  
Vancouver, Canada

Date \textit{August 24, 2001}
ABSTRACT

The present series of experiments was designed to investigate the potential inhibitory influence of stress and stress-related hormones on the 5-HT$_{2A}$ receptor-mediated behavioural effects of risperidone, an atypical antipsychotic. Experiment 1 examined the effects of chronic risperidone treatment; Long-Evans male rats were assigned to a daily regimen of either corticosterone (20 mg/kg) or vehicle for 14 days. Risperidone was administered at a dose of 0.1 mg/kg for 14 days prior to testing. Rats were tested on measures of sexual behaviour and wet dog shakes (WDS), both 5-HT$_{2A}$ receptor-mediated behaviours. Consistent with previous findings, chronic corticosterone treatment induced behavioural changes indicative of an increase in 5-HT$_{2A}$ receptor activity. Results from Experiment 1 demonstrated the efficacy of risperidone in facilitating rat sexual behaviour and inhibiting WDS frequency. Of interest, the behavioural effects of risperidone were significantly attenuated by a chronic corticosterone regimen. In Experiment 2, the behavioural influence of chronic mild stress (CMS) on a chronic risperidone regimen was examined. Rats received daily injections of either risperidone (0.1 mg/kg) or saline and were exposed daily to either CMS or no stress for 21 days. Similar to findings using a chronic corticosterone regimen, CMS significantly attenuated the effects of risperidone on both male rat sexual behaviour and WDS expression. Experiment 3 investigated the possibility that chronic CMS treatment act via elevated corticosterone levels when influencing the behavioural efficacy of risperidone. Rats received risperidone and metyrapone (50 mg/kg), a corticosterone synthesis inhibitor, on a daily basis for a period of 14 days. Results from Experiment 3 indicated the effects of stress on WDS frequency, but not on sexual behaviour, in risperidone-treated rats are likely mediated by corticosterone. Taken together, the data implicate both corticosterone and stress as playing an inhibitory role on the behavioural efficacy of risperidone. The current
findings may help to explain the high incidence of psychotic patients who are non-responsive to their medications and demonstrate elevated levels of stress-related hormones.
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INTRODUCTION

The emergence of atypical antipsychotic medications marked a shift in therapeutic focus from the dopaminergic to the serotonergic (5-HT) neurotransmitter system; agents acting on the 5-HT system have demonstrated improved efficacy in treating patients with psychotic disorders (Breier, 1995; Meltzer, 1999). Within the 5-HT system, numerous receptor subtypes exist (for review see Murphy et al., 1998). Among the various 5-HT receptor subtypes, antipsychotic medications that influence 5-HT$_{2A}$ receptors have been the most promising (e.g., Kuoppamaki, Palvimaki, Hietala, & Syvalahti, 1995; Schreiber, Brocco, Millan, 1994). More specifically, 5-HT$_{2A}$ receptor antagonism has been associated with a greater reduction in positive (e.g., hallucinations and delusional thinking) and negative (e.g., anergy, apathy, and social withdrawal) psychotic symptoms, a lower incidence of extrapyramidal side effects (EPS) and improved cognitive functioning (Breier, 1995; Chouinard et al., 1993; Schmidt, Sorenson, Kehne, Carr & Palfreyman, 1995).

On the forefront of therapeutic efficacy is the atypical antipsychotic risperidone (Megen et al., 1994; Song, 1997). Randomized, placebo-controlled, double-blind studies have indicated the superiority of risperidone to conventional antipsychotic medications (e.g., Chouinard et al., 1993; Claus et al., 1992). When compared to other atypical antipsychotics (e.g., clozapine), risperidone has demonstrated a greater tolerability and lower side effect profile (Heinrich, Klieser, Lehmann & Kinzler, 1991). Risperidone primarily acts as a 5-HT$_{2A}$ receptor antagonist and has higher affinity for 5-HT$_{2A}$ receptors than any other clinically approved antipsychotic medication (Breier, 1995; Canton, Verriele & Millan, 1994; Megens et al., 1994). Although, at higher doses, risperidone displays $D_2$ receptor antagonism, its clinical efficacy has been attributed to 5-HT$_{2A}$ receptor blockade (Megens et al., 1994; Meltzer, 1999). For example, doses
higher than 10 mg/day are associated with an increase in EPS that are characteristic of D2 receptor antagonism (Breier, 1995). At the recommended dosages, risperidone (4 mg/day) and other 5-HT2A receptor selective antipsychotics produce fewer EPS than haloperidol, a conventional D2 receptor antipsychotic (Megens et al., 1994; Meltzer, 1999). Moreover, PET studies of dopamine receptor occupancy have suggested that high 5-HT2A receptor occupancy (relative to D2 receptor occupancy) is necessary to avoid EPS with these agents (Meltzer, 1999).

Research with animals has also indicated the predominance of 5-HT2A receptor antagonism by risperidone, at low doses. In the rat, ex vivo autoradiography studies have demonstrated that doses between 0.037 and 0.12 mg/kg induce 50% receptor occupancy in the frontal cortex and maximal change in 5-HT2A receptor-mediated behaviours (Schotte, Bonaventure, Janssen, & Leysen, 1995; Schotte, Bruyckere, Janssen, & Leysen, 1989). In contrast, behavioural changes associated with D2 receptor antagonism and 50% D2 receptor occupancy do not occur until doses of 0.75 mg/kg to 2.5 mg/kg (Schotte et al., 1995; Schotte et al., 1989). Unlike other atypical antipsychotics, the relatively slow shift from 5-HT2A receptor occupancy to D2 receptors allows for the study of risperidone's influence on 5-HT2A receptors (and its associated behaviours) in isolation.

Recent advances in behavioural pharmacology have helped delineate the complexities of the 5-HT neurotransmitter system and the different behaviours influenced by its specific receptor subtypes. Unique to the 5-HT2A receptor is its affect on a 5-HT behavioural stereotypy known as wet dog shakes (WDS). WDS can be described as a reflexive shudder of the head, neck and trunk and can be induced pharmacologically with 5-HT2A receptor agonists in both rats and mice (Goodwin, Green, Johnson, 1984; Pranzatelli, 1990). For example, central administration of the 5-HT2A receptor agonist (+) 1- (2,5 dimethyl-4-iodophenyl)-2-aminopropane (DOI) into the
ventromedial brainstem (Watson & Gorzalka, 1992) or the prefrontal cortex (Willins & Meltzer, 1997) of male rats produces a dose-dependent increase in WDS. Moreover, risperidone and other 5-HT$_{2A}$ receptor antagonists inhibit 5-HT$_{2A}$ receptor agonist-induced WDS in a dose-dependent manner (Barwick, Jones, Richter, Hicks & Young, 2000; Janssen et al., 1988; Pranzatelli, 1990; Wettstein, Host & Hitchcock, 1999). Administration of agonists and antagonists specific to other 5-HT receptor subtypes do not produce such an effect (Pranzatelli, 1990). Measurement of WDS frequency has gained acceptance as a non-invasive behavioural assay of central 5-HT$_{2A}$ receptor activity (Eison, Freeman, Guss & Mullins, 1995; Gorzalka & Hanson, 1998; Kuroda, Mikuni, Ogawa & Takahashi, 1992, Watson & Gorzalka, 1990; 1992; Yap & Taylor, 1983).

Also influenced by the 5-HT$_{2A}$ receptor is male rat sexual behaviour (e.g., Foreman, Hall & Love, 1989). Systemic administration of selective 5-HT$_{2A}$ receptor antagonists can facilitate male rat sexual behavior (Abraham, Viesca, Plaza & Marin, 1988). Similarly, both chronic and acute administration of risperidone, at doses relatively selective to the 5-HT$_{2A}$ receptor, induce an increase in male rat sexual activity (Drago et al., 1997; Genazzani, Mauceri, Valerio, Nardo & Drago, 1990). Consistent with an increase in 5-HT$_{2A}$ receptor activity, central administration of DOI to male rats produces a dose-dependent inhibition of sexual behaviour; 5-HT$_{2A}$ receptor antagonists can reverse this DOI-induced sexual inhibition (Watson & Gorzalka, 1991). Taken together, the data suggest that 5-HT$_{2A}$ receptor activation mediates an inhibitory influence on male rat sexual behavior.

Thus, studies have implicated both the inhibition of male copulatory responses and the facilitation of WDS with increased 5-HT$_{2A}$ receptor activity. Further research has revealed a direct, negative relationship between these two behaviors in male rats: decreases in spontaneous
WDS are significantly correlated with increases in sexual behavior (Watson & Gorzalka, 1990). Additionally, the selective 5-HT$_{2A}$ receptor agonist, DOI, has been shown to magnify this inverse relationship (Watson & Gorzalka, 1990). The observed inverse correlation between WDS and copulatory proficiency can be attributed to overlapping neural substrates in the ventromedial brainstem (Watson & Gorzalka, 1992), suggesting that the two behaviors are neurally dependent.

An intimate, interactive relationship between hormones of the hypothalamic-pituitary adrenocortical (HPA) axis and the 5-HT neurotransmitter system has been demonstrated (for review see Chaouloff, 1995). The HPA axis becomes activated during times of stress and releases the adrenocortical steroid corticosterone. Recent studies have implicated corticosterone as playing a fundamental role in the regulation of 5-HT$_{2A}$ receptor density (e.g., Kuroda, Mikuni, Ogawa & Takahashi, 1992; McKittrick, Blanchard, Blanchard, McEwen & Sakai, 1995). More specifically, it has been demonstrated, using radioligand binding procedures, that chronic administration of corticosterone (at levels that mimic those seen during times of stress) can induce an increase in 5-HT$_{2A}$ receptor density in the rat brain, which is a direct indication of an increase in 5-HT$_{2A}$ receptor activity (e.g, Fernandes, McKittrick, File & McEwen, 1997; Kuroda, et. al., 1992). At a behavioural level, chronic corticosterone treatment (20 mg/kg or 50 mg/kg) induces a decrease in male rat sexual behaviour and concurrent increase in WDS (Gorzalka & Hanson, 1998). Administration of the antidepressant nefazadone, which possesses 5-HT$_{2A}$ receptor antagonistic properties, can attenuate the effects of corticosterone on rat sexual behaviour and WDS (Hanson, Gorzalka & Brotto, 1998). Thus, the behavioural data offer further evidence for a corticosterone-induced increase in 5-HT$_{2A}$ receptor activity. The effects of corticosterone on sexual behaviour and WDS may be attributed to its influence on 5-HT$_{2A}$ receptor density.
The inhibitory influence of 5-HT$_{2A}$ receptor activity have also been examined. Studies employing various chronic stress paradigms have demonstrated significant increases in serum corticosterone levels and increases in 5-HT$_{2A}$ receptors in the cerebral cortex; changes in 5-HT$_{2A}$ receptor density were proportional to the degree of HPA-axis activation and corticosterone secretion (e.g., McKittrick et al., 1995). Consistent with these findings, chronic stress has also been found to significantly increase WDS frequency (e.g., Gorzalka, Hanson & Brotto, 1998; Takao et al., 1995) and decrease male rat sexual behaviour (e.g., Brotto, Gorzalka & Hanson, 1998; Retana-Marquez, Salazar & Velazquez-Moctezuma, 1996). In sum, the demonstrated influence of corticosterone on 5-HT$_{2A}$ receptor activity can be extended to general HPA axis activation (via chronic stress procedures). It is unclear, however, if the behavioural influences observed are actually being mediated by the same pathway. Although changes in WDS behaviour appear to be influenced by a similar pathway, there is recent indication that the effects of stress on sexual behaviour may not be mediated by the same mechanism as corticosterone (Gorzalka, Hanson & Hong, *manuscript in preparation*). The apparent contradiction in the findings may be explained by the relative vulnerability of rat sexual behaviour to other (unspecified) factors, but further investigation is required.

The apparent influence of corticosterone on 5-HT$_{2A}$ receptor activity holds important implications for the effectiveness of risperidone as both a 5-HT$_{2A}$ receptor antagonist and an antipsychotic agent. Although it has been shown that 5-HT$_{2A}$ receptor antagonists can attenuate the 5-HT$_{2A}$ receptor-mediated behavioural effects of corticosterone (e.g., Hanson et al., 1998), the possibility that corticosterone may impede the efficacy of a 5-HT$_{2A}$ receptor antagonist alone has not been explored. There is recent indication that DOI pre-treatment can interfere with the behavioural effects of risperidone in the paw test, an established animal model of antipsychotic
efficacy (Ellenbroek, Prinssen & Cools, 1994). In the same way, corticosterone-induced changes in 5-HT$_{2A}$ receptor density may also have an inhibitory influence.

Within the human literature, links between psychotic disorders and HPA axis dysfunction have been made (e.g., Gispen-de Wied, 2000; Jansen, Gispen-de Wied, Gademan, Jonge, Linden, & Kahn, 1998; Pivac, Muck-Seler & Jakovljevic, 1997). It has been suggested that subsets of the psychotic patient population suffer from elevated levels of cortisol levels and that a high proportion of stressful life events predicts psychotic relapse (Gispen-de Wied, 2000; Jansen, Gispen-de Wied, Kahn, 2000). Given that both risperidone and HPA axis hormones influence the same receptor in opposing directions, investigation of the potential involvement of the HPA axis in affecting the pharmacological efficacy of risperidone is both scientifically and clinically relevant.

The present series of experiments was designed to satisfy the following three research objectives:

1) Research has indicated that chronic risperidone treatment, at a dose of 0.1 mg/kg or less, can effectively increase sexual behaviour (Drago et al., 1997) and block WDS frequency (Wettstein et al., 1999). By contrast, chronic corticosterone treatment (at doses that mimic levels seen during times of stress) induces an increase in 5-HT$_{2A}$ receptor activity, as demonstrated by a concurrent inhibition of male rat sexual behaviour and facilitation of WDS (e.g, Gorzalka & Hanson, 1998; Gorzalka, Brotto & Hong, 1999). Experiment 1 was designed to examine the possible inhibitory influence of corticosterone on the 5-HT$_{2A}$ receptor-mediated behavioural effects of chronic risperidone treatment.

2) Evidence has also implicated chronic stress (and subsequent HPA axis activation) as having similar influence as corticosterone on 5-HT$_{2A}$ receptor-mediated behaviours and
The inhibitory influence of $5\text{-HT}_{2A}$ receptor density (e.g., McKittrick et al., 1995; Gorzalka & Hanson, 1998). To the extent that stress mimics the $5\text{-HT}_{2A}$ receptor-mediated behavioural influence of corticosterone, Experiment 2 investigated the potential interference of stress on chronic risperidone treatment.

3) Although the $5\text{-HT}_{2A}$ receptor-mediated behavioural effects of chronic corticosterone and chronic stress treatments are similar, there is recent indication that these treatments may represent different mechanisms of influence on male rat sexual behaviour (Gorzalka et al., manuscript in preparation). To the extent that the behavioural results from objective 1 and 2 are similar, Experiment 3 incorporated the corticosterone synthesis inhibitor metyrapone into the chronic stress-risperidone paradigm. Findings from this study will help elucidate the potential role of corticosterone in the behavioural influence of stress on the pharmacological efficacy of risperidone.

**METHOD**

**Animals**

Animals were housed in groups of three or four in the standard triple wire mesh cages and were allowed free access to both Purina Rat Chow and water. The colony was maintained at a temperature of $21 \pm 1^\circ\text{C}$ and on a reverse 12/12 hour light/dark cycle (lights off at 0900h). Subjects were screened for copulatory proficiency; rats were required to ejaculate (at least once) within a 25 minute screening session. A maximum of 3 screening sessions was allowed.

Sexually-experienced female Wistar rats were employed as sexual behaviour stimuli. Females were bilaterally ovariectomized, at 3 months of age, while under a combination of xylazine (8 mg/kg) and ketamine (95 mg/kg) anesthesia. Females weighed approximately 500-600 g and were 8-14 months of age when used.
Drugs

Doses of estradiol benzoate (EB) and progesterone (P) (Sigma Chemical Company, St. Louis, MO) were dissolved in 0.1 ml peanut oil.

Corticosterone (Sigma Chemical Co., St. Louis, MO), dissolved in propylene glycol at dose of 20 mg/kg.

Risperidone (Sigma Chemical Co., St. Louis, MO), dissolved in 0.9% saline at a dose of 0.1 mg/kg.

(±) 1- (2,5 dimethyl-4-iodophenyl)-2-aminopropane (DOI; Sigma Chemical Co., St. Louis, MO), dissolved in 0.9% saline at a dose of 0.5 mg/kg.

Metyrapone (Sigma Chemical Company, St. Louis, MO) was dissolved in propylene glycol at a dose of 50mg/kg.

Apparatus

All animals were injected in plastic maternity bins with 26 gauge and one-half inch stainless steel needles. Rats were tested in cubical (30 x 30 x 45 cm) or cylindrical (30cm in diameter x 45cm in height) Plex-i-glas chambers; all chamber floors were lined with contact bedding. Latencies were timed with a stopwatch and behavioural measures were recorded on standard score sheets by trained observers who were blind to all treatment conditions.

Procedure

Prior to testing, females were injected with EB (10 μg) and P (500 μg) to induce sexual receptivity. EB was injected (sc) 44-48 hours prior to testing, while P was injected (sc) 3-4 hours prior to testing; 0.1 ml of each solution was administered.

Injections and behavioural testing were performed during the middle third of the dark
cycle. Subjects were allowed to habituate individually to a testing chamber for 5 minutes before testing began. Test sessions were 30 minutes in length and commenced upon the introduction of a stimulus female. Females were interchanged among the subjects every 10 minutes to maintain sexual interest.

The following sexual behaviour parameters were scored: 1) frequency of mounts with pelvic thrusting prior to ejaculation; 2) frequency of intromissions prior to ejaculation; 3) frequency of ejaculations; 4) mount latency, the time between the start of the test session and the first mount; 5) intromission latency, the time between the start of the test session and the first intromission; 6) ejaculation latency, the time between the first intromission and first ejaculation; 7) post-ejaculatory interval, the time between the first ejaculation and the first intromission of the next copulatory bout. The frequency of WDS was concomitantly recorded throughout the test session. Male rats that failed to ejaculate during the test session were dropped from the analyses of mounts and intromission frequencies and latency scores were set to the maximum of 1800 seconds. Rats that failed to intromit after the first ejaculation were dropped from the analyses of the post-ejaculatory interval.

EXPERIMENT 1

Effects of Chronic Risperidone and Corticosterone

The first experiment was designed to examine the behavioural influence of chronic corticosterone treatment on chronic risperidone administration. Both corticosterone and risperidone treatments, alone, have been shown to influence 5-HT$_{2A}$ receptor activity and 5-HT$_{2A}$ receptor-mediated behaviours (Drago et al., 1997; Gorzalka & Hanson, 1998; Kuroda et al., 1992). Given that chronic corticosterone treatment induces an increase in 5-HT$_{2A}$ receptor
density/activity (Kuroda et al., 1992), the potential inhibitory role of corticosterone on risperidone’s ability to antagonize the 5-HT2A receptor was investigated. WDS frequency and male rat sexual behaviour, both 5-HT2A receptor-mediated behaviours, were concomitantly scored to measure changes in 5-HT2A receptor activity.

**Animals**

Long-Evans male rats (n=20; Charles River Canada Inc., Montreal) were approximately 8 months old and between 600-800 g at the time of testing.

**Procedure**

Subjects were randomly divided into two equal groups (n = 10) and were administered (sc) either risperidone (RIS; 1 ml/kg) or saline once daily for a period of 10 days. On the 11th day, 30 minutes prior to testing, rats were administered DOI (1 ml/kg). Rats were tested on measures of sexual behaviour and WDS (TEST 1).

Two weeks following the test session, rats (n= 20) were re-assigned to the same treatment groups. In addition to the original treatment regimen, rats were administered corticosterone (CORT; 1 ml/kg). Rats, again, received daily injections (sc) for a duration of 10 days and were administered DOI (1ml/kg) 30 minutes prior to testing. Rats were re-tested on measures of sexual behaviour and WDS (TEST 2).

Data were analyzed for main and interaction effects using a repeated-measures Analysis of Variance (ANOVA); significance level was set at p < 0.05.

**Results and Discussion**

Data for WDS and sexual behaviour are presented in Table 1 (TEST 1) and Table 2 (TEST 2).
A significant main effect of CORT was indicated for measures of mount latency ($F(1,18) = 7.05, p < 0.016$), intromission latency ($F(1,18) = 8.03, p < 0.011$), ejaculation latency ($F(1,18) = 10.63, p < 0.004$), post-ejaculatory interval ($F(1,14) = 9.48, p < 0.008$), ejaculation frequency ($F(1,18) = 15.78, p < 0.001$), and WDS frequency ($F(1,18) = 19.59, p < 0.0001$). Changes were indicative of an increase in 5-HT$_{2A}$ receptor activity.

The observed main effect of RJS suggested that chronic RIS effectively increased sexual activity and blocked DOI-induced WDS. RIS shortened mount latency ($F(1,18) = 7.30, p < 0.015$), intromission latency ($F(1,18) = 7.83, p < 0.0012$), ejaculation latency ($F(1,18) = 11.78, p < 0.003$), post-ejaculatory interval ($F(1,18) = 35.64, p < 0.0001$), increased the frequency of ejaculations ($F(1,18) = 11.24, p < 0.004$) and lowered the frequency of DOI-induced WDS ($F(1,18) = 37.38, p < 0.0001$). Taken together, the results indicate the effectiveness of chronic RIS treatment in decreasing 5-HT$_{2A}$ receptor activity, as demonstrated by a facilitation of sexual behaviour and inhibition of DOI-induced WDS frequency.

An inhibitory influence of CORT on the behavioural effectiveness of RIS was suggested by significant interactions found on measures of sexual behaviour. When compared to RIS rats, rats treated with CORT-RIS showed significantly longer intromission latency ($F(1,18) = 4.21, p < 0.05$) and post-ejaculatory interval ($F(1,14) = 9.48, p < 0.008$) and lower frequency of ejaculations ($F(1,18) = 10.57, p < 0.004$). That is, CORT significantly blocked the RIS-induced facilitation of sexual behaviour. In the same way, trends toward significance ($p < 0.10$) were found on mount latency, ejaculation latency and WDS frequency measures; CORT inhibited the effects of RIS.

The current findings support previous findings that indicate chronic corticosterone treatment as increasing 5-HT$_{2A}$ receptor activity, as measured by an inhibition of sexual
behaviour and facilitation of WDS expression. The 5-HT$_{2A}$ receptor antagonistic properties of risperidone were also supported. Risperidone induced behavioural changes that suggested a decrease in 5-HT$_{2A}$ receptor activity- an increase in sexual behaviour and a decrease in WDS frequency. Of interest, chronic corticosterone treatment either attenuated or showed a trend to attenuate effects of risperidone on both WDS frequency and male rat sexual behaviour. The results indicate that corticosterone inhibits the 5-HT$_{2A}$ receptor antagonist activity of risperidone.

**EXPERIMENT 2**

**Effects of Chronic Risperidone and Stress**

Experiment 2 was designed to investigate the influence of chronic stress on the behavioural effects of a chronic risperidone regimen. Similar to a chronic corticosterone regimen, chronic stress has been shown to increase the frequency of WDS and decrease male rat sexual activity (e.g., Gorzalka, Hanson & Brotto, 1998). Also consistent with an increase 5-HT$_{2A}$ receptor activity, chronic stress can induce an upregulation of 5-HT$_{2A}$ receptors (e.g., McKittrick et al., 1995; Takao et al., 1995). Adapting the chronic mild stress (CMS) procedure outlined in Kopp et al. (1999), the present study examined the possible generalization of the inhibitory role of chronic corticosterone treatment (Experiment 1) to stress.

**Animals**

Long-Evans male rats (n=34; Charles River Canada Inc., Montreal) were 6 months old at the time of testing and weighed between 450 and 700g.

**Apparatus**

The strobe light/white noise stressor was conducted in a black wooden box (100cm x 100cm x 80cm ht.) and used a portable strobe light and radio; the floor of the apparatus was
generously lined with contact bedding. For the tube confinement stressor, 20cm long x 6cm diameter plastic tubes were used. Tubes were open at one end and covered at the other with a plex-I-glas square (5cm x 5cm) with a circular air hole (1 cm dia.) in the center. Plastic maternity bins with single wire mesh lids, lined with damp contact bedding, were used in the wet bedding stressor.

Procedure

Males were randomly assigned to one of the following four groups: 1) saline and stress (n=7; STRESS); 2) saline and no stress (n=8; NO STRESS); 3) risperidone and stress (n=9; RIS-STRESS); 4) risperidone and no stress (n=10; RIS). Risperidone or saline was injected (sc) at a volume of 1 ml/kg, once daily for a period of 21 days. Rats assigned to one of the two stress conditions were exposed to a 21-day chronic stress schedule. The schedule was adapted from that outlined by Kopp and colleagues (1999) and included the following stressors: 1) tube confinement (1h) in which movement was restricted; 2) food and water deprivation (15h); 3) food restriction (50g/rat; 2h) after food deprivation; 4) empty water bottles after water deprivation (2h); 5) stroboscopic illumination/white noise (30 min.); 6) wet bedding/isolation (200ml water/100g bedding; 18h); 7) cage (mate) rotation (4h); 8) reversal of colony light/dark cycle (48h). Stressors varied daily to minimize habituation.

Prior to stress and injection exposure, rats underwent sexual and WDS behaviour testing to provide a baseline measure. Rats were tested on measures of sexual behaviour and WDS frequency every seven days; the last test session occurred on Day 22 of the experiment. On each test day, rats were not exposed to a morning stressor and injections were administered following behavioural testing.
Data, collected across the 3 weeks, were analyzed using a repeated-measures ANOVA. Weekly differences were analyzed using a one-way ANOVA; all parameters that reached significance were further analyzed using the Least Significant Difference (LSD) multiple comparisons test. The significance criterion was maintained at 0.05.

**Results and Discussion**

Significant behavioural differences were not found at baseline. Weekly data for all measures are presented in Table 2 and weekly data for WDS frequency and ejaculation latency are presented in Figures 1 and 2, respectively.

Repeated measures ANOVA indicated treatment conditions varied significantly by week on mount latency \((F(1,30) = 4.95, p \leq 0.034)\), intromission latency \((F(1,30) = 5.77, p \leq 0.023)\) and ejaculation frequency \((F(1,30) = 3.90, p \leq 0.05)\) measures. A main effect of treatment condition was found on mount latency \((F(3,30) = 6.34, p \leq 0.002)\), intromission latency \((F(3,30) = 6.34, p \leq 0.002)\), ejaculation latency \((F(3,30) = 11.57, p \leq 0.0001)\), post-ejaculatory interval \((F(3,14) = 10.86, p \leq 0.001)\), ejaculation frequency \((F(3,30) = 11.49, p \leq 0.0001)\) and WDS frequency \((F(3,30) = 11.51, p \leq 0.0001)\) measures. Significant interactions among test weeks and treatment conditions were not found.

Using one-way ANOVAs, differences among treatment conditions at each week were analyzed. At week 1, significant differences were found on the following measures: mount latency \((F(3,30) = 5.61, p \leq 0.004)\), intromission latency \((F(3,30) = 5.06, p \leq 0.006)\), ejaculation latency \((F(3,30) = 10.03, p \leq 0.0001)\), post-ejaculatory interval \((F(3,23) = 8.50, p \leq 0.001)\), ejaculation frequency \((F(3,30) = 5.46, p \leq 0.004)\) and WDS frequency \((F(3,30) = 3.54, p \leq 0.026)\). At week 2, significant differences were found on the following measures: mount latency \((F(3,30) = 3.37, p \leq 0.031)\), intromission latency \((F(3,30) = 3.57, p \leq 0.026)\), ejaculation
latency ($F(3,30) = 9.21, p < 0.0001$), post-ejaculatory interval ($F(3,25) = 7.98, p \leq 0.001$), ejaculation frequency ($F(3,30) = 9.07, p < 0.0001$) and WDS frequency ($F(3,30) = 5.75, p \leq 0.003$). At week 3, significant differences were found on the following measures: mount latency ($F(3,30) = 3.19, p \leq 0.038$), intromission latency ($F(3,30) = 3.33, p \leq 0.033$), ejaculation latency ($F(3,30) = 4.83, p \leq 0.007$), post-ejaculatory interval ($F(3,22) = 5.30, p \leq 0.007$), ejaculation frequency ($F(3,30) = 5.98, p \leq 0.003$) and WDS frequency ($F(3,30) = 11.54, p < 0.0001$). At each week, significant differences on measures of mount and intromission frequencies were not found at ($p > 0.05$).

Using the LSD multiple comparisons test, significant differences between treatment conditions were explored, at each week.

CMS significantly decreased sexual activity from weeks 1-3; these effects strengthened with increased CMS exposure. At week 1, STRESS rats, when compared with NO STRESS rats, demonstrated increased ejaculation latency ($p \leq 0.01$) and post ejaculatory interval ($p \leq 0.03$). At week 2, the increase in ejaculation latency ($p \leq 0.003$) and post-ejaculatory interval ($p \leq 0.006$) was maintained. At week 3, in addition to the increase the ejaculation latency ($p \leq 0.008$) and post-ejaculatory interval ($p \leq 0.02$), the frequency of ejaculations decreased ($p \leq 0.03$). CMS also effectively increased WDS frequency at week 1 ($p \leq 0.03$), week 2 ($p \leq 0.01$) and week 3 ($p \leq 0.001$).

Chronic administration of risperidone, when compared to control, facilitated copulatory behaviour, but only reached significance at weeks 2 and 3. More specifically, risperidone significantly shortened the latency to ejaculate at both week 2 ($p \leq 0.02$) and week 3 ($p \leq 0.002$). Although significant differences in WDS behaviour between RIS rats and NO STRESS rats were
not revealed, RIS rats demonstrated significantly lower WDS frequency when compared with STRESS rats at week 1 ($p \leq 0.005$), week 2 ($p \leq 0.010$) and week 3 ($p \leq 0.0001$).

Significant differences were not found among rats exposed to CMS; risperidone did not attenuate the effects of CMS on sexual behaviour and WDS frequency ($p > 0.05$).

Comparison of RIS rats to RIS-STRESS rats revealed significant differences on parameters of sexual behaviour and WDS frequency. At week 1, RIS-STRESS animals showed significantly higher mount latencies ($p \leq 0.001$), intromission latencies ($p \leq 0.002$), ejaculation latencies ($p < 0.0001$), post-ejaculatory intervals ($p < 0.0001$) and lower ejaculation frequencies ($p \leq 0.001$). At week 2, RIS-STRESS significantly increased intromission latency ($p \leq 0.004$), ejaculation latency ($p < 0.0001$), post-ejaculatory interval ($p < 0.0001$) and decreased ejaculation frequency ($p < 0.0001$). In addition, RIS-STRESS significantly increased WDS frequency ($p \leq 0.004$). At week 3, significantly longer post-ejaculatory intervals ($p \leq 0.01$) and ejaculation latencies ($p \leq 0.03$), lower ejaculation frequencies ($p \leq 0.005$) and higher WDS frequencies ($p < 0.0001$) were found in RIS-STRESS rats. Taken together, stress significantly attenuated the facilitatory effects of risperidone on copulatory behaviour at weeks 1, 2 and 3 and blocked risperidone's inhibitory effect on WDS frequency at weeks 2 and 3.

The present findings are consistent with results found in Experiment 1. Similar to chronic corticosterone administration, CMS exposure significantly inhibited sexual behaviour and facilitated WDS expression. Risperidone effectively increased sexual activity and decreased WDS expression, which is consistent with its properties as a 5-HT$_{2A}$ receptor antagonist. CMS exposure also mimicked the inhibitory effects of corticosterone on risperidone; stress significantly attenuated the risperidone-induced changes in 5-HT$_{2A}$ receptor-mediated behaviours.
EXPERIMENT 3
Effects of Chronic Metyrapone, Risperidone and Stress

Results from Experiment 1 and 2 suggest that both chronic corticosterone and stress treatments act to inhibit the efficacy of risperidone as a 5-HT$_{2A}$ receptor antagonist. Experiment 3 was designed to investigate the possibility that the behavioural effects observed in both Experiments 1 and 2 can be attributed to corticosterone. The current study incorporated the corticosterone synthesis inhibitor metyrapone into a 14-day CMS-risperidone paradigm.

Animals

Long-Evans male rats (n = 39; Charles River Canada Inc., Montreal) were approximately 8-14 months in age and weighed between 515-800 g at the time of testing.

Apparatus

Stressors used are outlined in Experiment 2.

Procedure

Male rats were randomly assigned to one of four treatment groups: 1) saline (1ml/kg), metyrapone (2 ml/kg) and stress (n = 10; STRESS); 2) saline (1ml/kg), metyrapone (2ml/kg) and no stress (n=10; NO STRESS); 3) risperidone (1 ml/kg), metyrapone (2 ml/kg) and stress (n=9; RIS-STRESS); 4) risperidone (1 ml/kg), metyrapone (2 ml/kg) and no stress (n=10; RIS). Injections (sc) were given once daily for a period of 14 days and behavioural testing occurred 24 hours after the last injection. A baseline test was performed one week prior to the first test day. The last test session occurred on day 15 of the experiment. The stress schedule from Experiment 2 was implemented, with the exception of using only the first 14 days of the 21-day schedule. On each test day, rats were not exposed to a morning stressor and injections were administered following behavioural testing.
Data, collected across the 2 weeks, were analyzed using a repeated-measures ANOVA. Weekly differences were analyzed using a one-way ANOVA; all parameters that reached significance were further analyzed using the Least Significant Difference (LSD) multiple comparisons test. The significance criterion was maintained at 0.05.

Results and Discussion

At baseline, significant differences among treatment groups were not observed. Weekly data for all measures are presented in Table 3 and weekly WDS frequency data are presented in Figure 3.

A repeated-measures ANOVA indicated a significant main effect of time. Changes among treatment conditions varied significantly by week on the following measures: mount latency ($F(1, 35) = 42.04$, $p < 0.0001$), intromission latency ($F(1, 35) = 46.22$, $p < 0.0001$), ejaculation latency ($F(1, 35) = 36.40$, $p < 0.0001$), post-ejaculatory interval ($F(1, 26) = 35.63$, $p < 0.0001$), ejaculation frequency ($F(1, 35) = 47.47$, $p < 0.0001$) and WDS frequency ($F(1, 35) = 8.56$, $p < 0.006$). The following measures indicated a significant main effect of treatment condition: mount latency ($F(3, 35) = 7.45$, $p \leq 0.001$), intromission latency ($F(3, 35) = 6.92$, $p \leq 0.001$), ejaculation latency ($F(3, 35) = 3.01$, $p \leq 0.05$), post-ejaculatory interval ($F(3, 26) = 4.70$, $p \leq 0.009$), ejaculation frequency ($F(3, 35) = 3.93$, $p \leq 0.016$) and WDS frequency ($F(3, 35) = 2.93$, $p \leq 0.05$). Significant interaction effects were not found.

At week 1, as revealed by the one-way ANOVA, the effects of metyrapone were not apparent and results replicated those found in Experiment 2. Significant differences were found on the following measures: mount latency ($F(3, 35) = 4.36$, $p \leq 0.01$), intromission latency ($F(3, 35) = 5.01$, $p \leq 0.005$), post-ejaculatory interval ($F(3, 31) = 3.21$, $p \leq 0.04$), ejaculation frequency ($F(3, 35) = 3.50$, $p \leq 0.03$) and WDS frequency ($F(3, 35) = 5.31$, $p \leq 0.004$).
The LSD multiple comparisons test indicated that STRESS significantly inhibited sexual behaviour; STRESS increased mount latency \((p \leq 0.005)\), intromission latency \((p \leq 0.002)\), ejaculation latency \((p \leq 0.04)\), and the post-ejaculatory interval \((p \leq 0.01)\) and decreased in ejaculation frequency \((p \leq 0.01)\). Significant differences between STRESS and STRESS-RIS were not found. STRESS-RIS significantly increased mount latency \((p \leq 0.006)\), intromission latency \((p \leq 0.002)\), ejaculation latency \((p \leq 0.04)\), the post-ejaculatory interval \((p \leq 0.01)\) and WDS expression \((p \leq 0.003)\) and decreased ejaculation frequency \((p \leq 0.01)\).

RIS decreased WDS frequency \((p \leq 0.05)\) and post-ejaculatory interval length \((p \leq 0.05)\), suggesting an inhibition of WDS and facilitation of sexual behaviour. When compared to RIS, STRESS-RIS significantly increased the number of WDS \((p \leq 0.001)\) and decreased ejaculation latency \((p \leq 0.05)\). Thus, metyrapone failed to attenuate the effects of stress on risperidone, as indicated by the increase in WDS frequency and ejaculation latency.

At week 2, metyrapone significantly blocked the effects of stress on WDS frequency; significant differences among treatment conditions were not observed \((F(3, 35) = 0.854, p > 0.05)\). But, differences were maintained on mount latency \((F(3, 35) = 3.814, p \leq 0.02)\), intromission latency \((F(3, 35) = 3.719, p \leq 0.04)\) and the post-ejaculatory interval \((F(3, 29) = 3.567, p \leq 0.03)\). More specifically, STRESS continued to increase the length of mount \((p \leq 0.006)\) and intromission \((p \leq 0.01)\) latencies and the post-ejaculatory interval \((p \leq 0.008)\).

The present study replicated the STRESS results of Experiment 2, when the effects of metyrapone, a corticosterone synthesis inhibitor, were not apparent (i.e., week 1). The observed decrease in sexual activity and increase in WDS from CMS exposure is consistent with behavioural findings linked to corticosterone-induced increases in 5-HT2A receptor density (e.g., Gorzalka & Hanson, 1998; Kuroda, 1992). Following the second week of metyrapone treatment,
metyrapone effectively blocked the stress-induced increase in WDS; significant differences between STRESS-RIS and RIS rats in WDS expression disappeared. Thus, the data suggest that the facilitatory influence of stress on the expression of WDS can be attributed to elevated levels of corticosterone. But, metyrapone treatment only partially blocked the inhibitory effect of CMS on sexual behaviour. The effects of stress on corticosterone levels and sexual behavior do not appear to be directly related. Several other stress-induced hormonal and neural changes may account for the inhibition in male rat sexual behaviour.

**GENERAL DISCUSSION**

The current series of experiments was designed to investigate and satisfy the following three experimental objectives:

First, the potential inhibitory influence of chronic corticosterone treatment on the effectiveness of risperidone as a 5-HT$_{2A}$ receptor antagonist was examined. Recent research has suggested that chronic corticosterone administration induces an upregulation of 5-HT$_{2A}$ receptors and behavioural changes that are indicative of an increase in 5-HT$_{2A}$ receptor activity (Gorzalka & Hanson, 1998; Kuroda et al., 1992). Moreover, pretreatment with DOI can inhibit the efficacy of risperidone in the paw test (Ellenbroek et al., 1994). Given that risperidone acts to decrease 5-HT$_{2A}$ receptor activity, corticosterone-induced changes in 5-HT$_{2A}$ receptor density may also attenuate the pharmacological efficacy of risperidone. In Experiment 1, chronic corticosterone administration inhibited risperidone’s ability to influence both WDS and male rat sexual behaviour. More specifically, the addition of corticosterone to chronic risperidone treatment inhibited male rat sexual behaviour and facilitated WDS expression. Taken together, systemic alteration of basal corticosterone levels can affect the behavioural efficacy of
risperidone; corticosterone's ability to inhibit the risperidone-induced facilitation of sexual behaviour and inhibition of WDS is likely mediated by an upregulation of 5-HT$_{2A}$ receptors.

Second, the effects of a chronic stress paradigm in conjunction with a chronic risperidone regimen were explored. During times of stress the HPA axis becomes activated and releases corticosterone and other adrenocortical hormones. Ottenweller and colleagues (1992) argue that a state of 'chronic stress' is achieved when prolonged stress exposure induces sustained corticosterone elevation. It is not surprising that chronic corticosterone administration (at doses that mimic levels seen during times of chronic stress) produces the same 5-HT$_{2A}$ receptor-mediated behavioural changes as chronic stress exposure (e.g., Gorzalka & Hanson, 1998; Gorzalka et al., 1998). It has been shown that chronic stress induces a concomitant inhibition of sexual behaviour and facilitation of WDS expression (Brotto et al., 1998; Gorzalka et al., 1998). Moreover, 5-HT$_{2A}$ receptor density changes that are characteristic of chronic corticosterone administration have also been found following chronic stress exposure (McKittrick et al., 1995; Takao et al., 1995). To the extent that chronic stress exposure and chronic corticosterone treatment have similar 5-HT$_{2A}$ receptor-mediated behavioural influences, chronic stress exposure may, also, act to inhibit risperidone's pharmacological effects. Consistent with this hypothesis, Experiment 2 demonstrated the effectiveness of CMS in blocking risperidone's ability to facilitate sexual behaviour and inhibit WDS frequency.

Third, the possibility that the similar behavioural findings of Experiment 1 and 2 can be attributed to elevated levels of corticosterone was investigated. Following the chronic administration of metyrapone (a corticosterone synthesis inhibitor) the inhibitory effects of chronic stress exposure on risperidone for WDS frequency disappeared. With respect to WDS behaviour, it appears that the effects of stress on risperidone are likely mediated by
corticosterone elevation. In contrast, the inhibitory effects of stress on male rat sexual behaviour were only partially blocked by chronic metyrapone treatment. These findings are consistent with recent evidence that suggests the effects of stress on male rat sexual behaviour cannot be completely explained by a corticosterone-mediated mechanism (Gorzalka et al., *manuscript in preparation*).

Evidence for a link between the HPA axis and the 5-HT$_{2A}$ receptor is convincing. Both chronic corticosterone treatment and various chronic stress procedures induce an increase in 5-HT$_{2A}$ receptor density in the rat (e.g., Fernandes et al., 1997; Kuroda et al., 1992; McKittrick et al., 1995). Similarly, the serotonergic stereotypy WDS, identified as being unique to the 5-HT$_{2A}$ receptor (e.g., Eison et al., 1995; Watson & Gorzalka, 1990; Yap & Taylor, 1983) is facilitated upon either chronic corticosterone treatment or chronic stress exposure (Gorzalka & Hanson; Gorzalka et al., 1998; Takao et al., 1995). Although sexual behaviour is affected by several different 5-HT receptor subtypes, concomitant measurement of male rat sexual behaviour and WDS expression offers a reliable behavioural assay of 5-HT$_{2A}$ receptor activity (Brotto et al., 1998; Gorzalka & Hanson, 1998; Gorzalka et al., 1999; Watson & Gorzalka, 1990). Both chronic corticosterone treatment and chronic stress exposure induce a concurrent inhibition of male rat sexual behaviour and facilitation of WDS (Gorzalka & Hanson, 1998; Gorzalka et al., 1998; Gorzalka et al., 1999). Consistent with previous findings, results from both Experiments 1 and 2 suggested that chronic corticosterone and stress regimens act to increase 5-HT$_{2A}$ receptor activity, as indicated by a significant increases in WDS frequency and decreases in male rat sexual activity. Taken together, the data suggest the HPA axis as playing an important role in expression of 5-HT$_{2A}$ receptor-mediated behaviours.
Presumably, the similarity in behavioural findings of both chronic stress and corticosterone regimens suggests corticosterone as mediating these changes through the upregulation of 5-HT$_{2A}$ receptors. Similar to an adrenalectomy, chronic metyrapone treatment effectively blocks the ability of various stressors to induce higher plasma levels of corticosterone and related behavioural changes (e.g., Deroche, Piazza, Casolini, LeMoal & Simon, 1993; Reid, Ho, Tolliver, Wolkowitz & Berger, 1998). Findings from Experiment 3 indicated that the 5-HT$_{2A}$ receptor-mediated behavioural effects of stress cannot be entirely attributed to corticosterone elevation; inhibition of corticosterone elevation failed to (completely) block the effects of stress on the risperidone-induced changes in copulatory behaviour. Other animal behaviour models support the complexity of stress and its behavioural and physiological effects. For example, the stress-induced response to amphetamine treatment is only partially blocked by a chronic metyrapone regimen (Reid et al., 1998). More specifically, in stressed animals, metyrapone decreases locomotor sensitization to amphetamine treatment but increases the dopamine-releasing effect of amphetamine (Reid et al., 1998). Despite the reduction in stress-induced behaviours, the augmentation of amphetamine-induced dopamine release indicates the potential for an exacerbation (vs. alleviation) of psychotic symptoms and EPS. In sum, corticosterone appears to play a fundamental role in the expression of particular, but not all, stress-induced responses. To the extent that stress has an inhibitory influence on antipsychotic activity, the contribution of plasma corticosterone elevation remains equivocal.

Stress and corticosterone-induced alterations in 5-HT$_{2A}$ receptor density have been shown to be (brain) region specific (McKittrick et al., 1995; Kuroda et al., 1995; Takao et al., 1995). Using the radiolabelled ligand [3-H]ketanserin, chronic corticosterone administration has been shown to induce an increase in 5-HT$_{2A}$ receptor density in the frontal neocortex of the rat.
Chronic stress exposure also alters cortical 5-HT$_{2A}$ receptor density (McKittrick et al., 1995; Takao et al., 1995). For example, a forced swim stressor induces upregulation of 5-HT$_{2A}$ receptors in the rat frontal cortex (Takao et al., 1995), while psychosocial stressors are associated with 5-HT$_{2A}$ receptor upregulation in the parietal cortex (McKittrick et al., 1995). In addition, there is indication that an adrenalectomy only alters 5-HT$_{2A}$ receptor density in select brain regions. To date, no radioligand binding studies have examined the effects of corticosterone or stress in other brain regions. The standing possibility that corticosterone and stress differentially alter 5-HT$_{2A}$ receptor density in distinct brain regions may help to explain why chronic metyrapone treatment failed to block stress-induced changes in sexual behaviour. It has been suggested that the sexual behaviour effects of stress and corticosterone are mediated by two different mechanisms (Gorzalka et al., manuscript in preparation) and that the corticosterone-induced changes in 5-HT$_{2A}$ receptor density observed following a chronic stress regimen may not be sufficient in altering sexual activity.

Across all three studies risperidone, when administered alone, demonstrated an ability to facilitate sexual behaviour and inhibit WDS expression; an effect that was strengthened following a chronic injection regimen. The consistency of these behavioural results offers confidence in risperidone’s ability to act as a potent 5-HT$_{2A}$ receptor antagonist, at low doses. Studies employing selective 5-HT$_{2A}$ receptor antagonists LY 281067 and LY 53857 also demonstrate a facilitation of male rat sexual behaviour (Foreman et al., 1989). The current findings are also in agreement with the existing literature (e.g., Drago et al., 1997; Genazzani et al., 1990; Wettstein et al., 1999). For example, Drago and colleagues (1997) found that both chronic and acute administration of risperidone facilitated male rat sexual behaviour, but only at doses equivalent to 0.1 mg/kg; higher doses had no effect. Ex-vivo autoradiography studies have
shown the selectivity of risperidone to 5-HT2A receptors at low doses and the gradual shift in receptor occupancy to D2 receptors at higher doses (Schotte et al., 1995; Schotte et al., 1989). The fact that significant changes in male rat sexual behaviour are not found at higher doses is consistent with the receptor occupancy profile of risperidone (Drago et al., 1997). Conventional antipsychotics (e.g., haloperidol) primarily act as D2 receptor antagonists and act to inhibit male rat sexual behaviour (Drago et al., 1997). Therefore, at higher doses, the shift to D2 receptor occupancy by risperidone likely cancels its facilitatory effect on male rat copulatory behaviour (Drago et al., 1997). Taken together, the results from the present series of experiments suggest the facilitation of sexual behaviour (and inhibition of WDS) by risperidone may be explained by its influence on 5-HT2A receptors.

Recent evidence supporting the relevance of 5-HT2A receptors in antipsychotic drug action has also been demonstrated through various animal models of psychosis. Administration of phencyclidine (1(1-phenylcyclohexyl)piperidine hydrochloride; PCP), which can induce both positive and negative psychotic symptoms in humans, produces stereotyped behaviours and hyperlocomotion in rats (e.g., Castelli & Adams, 1981; Kitaichi, Yamada, Hasegawa, Furukawa & Nabeshima, 1994). Prevention of these behaviours has been linked to the therapeutic effects of different antipsychotic agents on both psychotic symptoms and EPS (e.g., Kitaichi et al., 1994). Risperidone-induced blockade of PCP-induced stereotyped behaviours has been primarily linked to its activity at the 5-HT2A receptor and presented as supportive evidence for its clinical efficacy in reducing EPS (Kitaichi et al., 1994). Similarly, selective 5-HT2A receptor antagonists have been found to be effective in blocking both amphetamine-induced (Schmidt et al., 1995) and PCP-induced locomotor activity (Gleason & Shannon, 1997) and demonstrating antipsychotic-like activity in the paw test (Ellenbroek et al., 1994). Noda and colleagues (1995)
recently demonstrated the ability of clozapine, ritanserin and risperidone (all selective to the 5-HT$_{2A}$ receptor) to attenuate the immobility effects of PCP in a forced swim test and the failure of haloperidol to produce such effects. It has been suggested that the PCP- forced swim test can be used as an effective animal model of negative psychotic symptoms (Noda, Yamada, Furukawa & Nabeshima, 1995). In sum, animal behaviour models of both 5-HT$_{2A}$ receptor activity and psychosis implicate the importance of 5-HT$_{2A}$ receptor antagonism in antipsychotic activity.

The 5-HT$_{2A}$ receptor antagonistic action of atypical antipsychotics have also been implicated in improving positive and negative symptoms of psychosis and EPS. Similar to conventional neuroleptics, double-blind, placebo-controlled studies have demonstrated the efficacy of risperidone (and other atypical antipsychotics) in reducing positive symptoms of chronic schizophrenia (e.g., Chouinard et al., 1993) One of the difficulties with conventional antipsychotic medications has been its relative ineffectiveness in treating negative psychotic symptoms (e.g., Breier, 1995). For example, chlorpromazine and high doses of loxapine, which produce high levels of D$_2$ receptor blockade in vivo, do not improve ameliorate negative symptoms (Meltzer, 1999). The recent advent of new, atypical antipsychotic has been promising: sertindole, ziprasidone, M100907, ritanserin and risperidone have all been found to be effective in treating negative symptoms (Breier, 1995; Megens et al., 1994; Meltzer, 1999). Various types of analyses to partial out the effect on negative symptoms from the effect on positive and depressive symptoms and EPS suggest that the effects of 5-HT$_{2A}$ receptor blockade on negative symptoms are direct (Tollefson & Sanger, 1996). Also unique to 5-HT$_{2A}$ receptor blockade has been the observed reduction in EPS when compared with conventional antipsychotic medications (Meltzer, 1999). For example, clinical trials of risperidone have shown that, at doses less than 6 mg/day, EPS were similar to placebo (Ellis, Cudkowicz, Sexton & Growdon, 2000). Moreover,
agents specifically developed for high 5-HT$_{2A}$ (and low D$_2$) receptor antagonist activity produce fewer EPS than haloperidol at comparable doses (for review see Meltzer, 1999). Taken together, the improved efficacy of atypical antipsychotics can, at least partially, be attributed to 5-HT$_{2A}$ receptor blockade.

Although risperidone, at low doses, acts as a selective antagonist to all 5-HT$_{2A}$ receptors, its mechanism of action appears to be highly localized to the frontal cortex (Megens et al., 1994). Using the radiolabelled ligand [3-H]ketanserin, risperidone demonstrates a high affinity for rat frontal cortex 5-HT$_{2A}$ receptors and is twice as potent as ritanserin, another highly selective 5-HT$_{2A}$ receptor antagonist, in this area (Megens et al., 1994). Also unique to risperidone is the lack of 5-HT$_{2A}$ receptor downregulation associated with a chronic risperidone regimen; chronic treatment of both conventional (i.e., chlorpromazine) and atypical (i.e., amperozide, clozapine and ORG 522) antipsychotics induce a significant decrease in 5-HT$_{2A}$ receptor binding sites (Kuppamaki et al., 1995). The results suggest that the 5-HT$_{2A}$ receptor antagonist action of risperidone cannot be explained by a downregulation of 5-HT$_{2A}$ receptors (Kuppamaki et al., 1995), and that risperidone may be more vulnerable to the 5-HT$_{2A}$ receptor upregulating effects of corticosterone and stress. Radioligand binding studies have demonstrated that corticosterone and stress-induced changes in 5-HT$_{2A}$ receptor density are specific to the cortical brain regions, particularly the rat frontal cortex (McKittrick et al., 1995; Kuroda et al., 1995; Takao et al., 1995). The implicated overlap of regional specificity of both risperidone and HPA axis hormones may also explain the observed inhibitory influence of stress and corticosterone on risperidone's pharmacological efficacy. Given that both HPA axis activation and risperidone primarily act to modify 5-HT$_{2A}$ receptor activity in the frontal cortex, it is
possible that this regional overlap is acting to enhance risperidone's susceptibility to the influence of HPA axis activation or dysfunction.

The current findings may be extended to the human literature. Three major lines of evidence are emerging that implicate the HPA axis in the development of and maintenance of psychotic disorders (Gispen-de Wied, 2000; Jansen et al., 1998; Pivac et al., 1997).

First, research has shown that schizophrenic patients are more sensitive to stressful life events and more likely to perceive events as stressful; this has been attributed to the lack of appropriate coping skills (e.g., Jansen et al., 2000; Yank, Bentley, Hargrove, 1993). Conversely, it has been shown that psychotic relapse can be predicted by stressful life events and the number of daily stressors experienced (Gispen-de Wied, 2000). Thus, a vicious circle develops. According to this line of research, psychotic patients are more vulnerable to chronic stress exposure and are more likely to prolong exposure with inadequate coping skills.

Second, examination of HPA axis function in psychotic patients has demonstrated elevated levels of cortisol and a blunted HPA axis response (Pivac et al., 1997; Muck-Seler, Pivac, Jakovljevic & Brzovic, 1999). For example, Muck-Seler and colleagues (1999) found that schizophrenic patients had significantly higher plasma cortisol levels and higher rates of non-suppression on the dexamethasone suppression test (DST) when compared to healthy subjects. Reports of diminished HPA axis response to various stressors in psychotic patients has also been attributed to hypercortisolemia (e.g., Jansen et al., 2000). In addition, chronic schizophrenic patients who demonstrate non-suppression on the DST show higher levels of plasma cortisol and score higher for negative symptoms (Kaneko et al., 1992). Elevated cortisol levels in psychotic patients are still found after controlling for the potential confound of depressive symptoms (Muck-Seler et al., 1999). Deliberate manipulation of cortisol levels are also associated with
schizophrenia-like symptoms. The degree of cortisol elevation in chronic schizophrenic patients, following a metachlorophenylpiperazine (m-CPP) challenge, has been associated with psychopathology severity; an increase in cortisol response was related to an increase in psychotic symptoms (Lindenmayer et al., 1997). Administration of exogenous corticosteroids, especially in high doses, can induce symptoms of mania, depression, paranoia, catatonia and delusional thinking (Abramovicz, 1993). Similarly, an excess of circulating glucocorticoids or HPA axis hormones has been associated with loss of receptors in the hippocampus, which is important in learning, emotion and memory (Young, Kwak & Kottak, 1995). Taken together, the data suggest subsets of the psychotic population suffer from HPA axis dysregulation, which has been associated with the degree of symptomology and severity.

Third, post-mortem studies of psychiatric and suicidal patients have shown increased 5-HT2A receptor density in the brain, particularly in the frontal cortex (Arora & Meltzer, 1989). The observed increase in 5-HT2A receptor density in the frontal cortex is consistent with evidence of corticosterone-induced density changes in the rat frontal cortex and may be explained by the reportedly elevated cortisol levels in psychotic patients. The current findings would suggest that the observed increase in 5-HT2A receptor density, particularly in the frontal cortex, would somehow impede the therapeutic efficacy of risperidone.

Although risperidone offers an improved therapeutic profile, relapse rates of up to 75% within 12 to 18 months after cessation of antipsychotic medications have been reported (Kane, 1999). Moreover, Kane (1999) reported that only 67% of risperidone-treated patients improved significantly and Remington and Kapur (2000) have documented the non-robustness of risperidone on refractory schizophrenic patients. The large number of patients who do not benefit
from risperidone may be indicative of a (unidentified) factor that is operating to hinder the effects of the drug.

To the extent that the clinical efficacy of risperidone is contingent upon its influence on the 5-HT$_{2A}$ receptor, an increase in 5-HT$_{2A}$ receptor density from HPA axis dysfunction would attenuate the therapeutic benefits of risperidone. Results from the current series of experiments suggest that HPA axis activation can inhibit the pharmacological efficacy of risperidone, via the 5-HT$_{2A}$ receptor. Given the growing evidence for both HPA axis dysfunction and potential lack of therapeutic efficacy of risperidone in subsets of the psychiatric population, the present findings may lend itself as a stepping stone toward elucidating the missing link between these two bodies of research.
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TABLE 1

EFFECTS OF CHRONIC CORTICOSTERONE AND CHRONIC RISPERIDONE
ON WDS AND SEXUAL BEHAVIOUR (MEAN ± SEM)

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<td>MOUNT LATENCY</td>
<td>1266.50 ± 271.6</td>
<td>198.70 ± 76.5</td>
</tr>
<tr>
<td>MOUNT FREQUENCY</td>
<td>20.0 ± 2.1</td>
<td>8.1 ± 1.8</td>
</tr>
<tr>
<td>INTROMISSION LATENCY</td>
<td>1306.70 ± 251.9</td>
<td>216.60 ± 75.5</td>
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<tr>
<td>INTROMISSION FREQUENCY</td>
<td>11.3 ± 2.9</td>
<td>11.9 ± 1.1</td>
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<tr>
<td>EJACULATION LATENCY</td>
<td>1422.50 ± 195.6</td>
<td>509.00 ± 86.5</td>
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<td>EJACULATION FREQUENCY</td>
<td>0.3 ± 0.15</td>
<td>1.70 ± 0.26</td>
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<tr>
<td>POST-EJACULATORY INTERVAL</td>
<td>1800 ± 0.00 *</td>
<td>419.30 ± 40.2</td>
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<tr>
<td>WDS FREQUENCY</td>
<td>10.2 ± 0.53</td>
<td>2.7 ± 1.6</td>
</tr>
</tbody>
</table>

* Post-Ejaculatory Intervals were set to a maximum of 1800 seconds when rats failed to ejaculate and were dropped from analyses when rats failed to intromit following the first ejaculation.
TABLE 2

WEEKLY EFFECTS OF CHRONIC STRESS AND CHRONIC RISPERIDONE
ON WDS AND SEXUAL BEHAVIOUR (MEAN ± SEM)

<table>
<thead>
<tr>
<th></th>
<th>VEHICLE</th>
<th>RIS</th>
<th>STRESS</th>
<th>STRESS-RIS</th>
</tr>
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<tbody>
<tr>
<td>MOUNT LATENCY</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>769.0 ± 267.6</td>
<td>303.9 ± 194.6</td>
<td>1402.0 ± 266.3</td>
<td>1461.1 ± 225.9</td>
</tr>
<tr>
<td>Week 2</td>
<td>544.1 ± 286.0</td>
<td>36.22 ± 14.1</td>
<td>828.0 ± 344.1</td>
<td>1099.7 ± 286.0</td>
</tr>
<tr>
<td>Week 3</td>
<td>432.9 ± 244.2</td>
<td>121.33 ± 60.3</td>
<td>1091.3 ± 337.4</td>
<td>871.60 ± 265.9</td>
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<tr>
<td>MOUNT FREQUENCY</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>11.5 ± 2.5</td>
<td>14.0 ± 1.4</td>
<td>18.0 ± 2.0</td>
<td>13.5 ± 5.5</td>
</tr>
<tr>
<td>Week 2</td>
<td>32.5 ± 12.8</td>
<td>11.22 ± 1.7</td>
<td>15.8 ± 3.2</td>
<td>20.5 ± 6.9</td>
</tr>
<tr>
<td>Week 3</td>
<td>19.3 ± 5.5</td>
<td>17.9 ± 2.9</td>
<td>34.7 ± 9.8</td>
<td>19.5 ± 3.9</td>
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<tr>
<td>INTROMISSION LATENCY</td>
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<tr>
<td>Week 1</td>
<td>794.5 ± 259.7</td>
<td>452.9 ± 207.9</td>
<td>1434.7 ± 245.9</td>
<td>1461.1 ± 225.9</td>
</tr>
<tr>
<td>Week 2</td>
<td>544.8 ± 285.8</td>
<td>69.33 ± 16.79</td>
<td>904.0 ± 322.4</td>
<td>1114.0 ± 280.4</td>
</tr>
<tr>
<td>Week 3</td>
<td>446.9 ± 241.9</td>
<td>181.33 ± 80.2</td>
<td>1143.0 ± 313.8</td>
<td>901.9 ± 259.4</td>
</tr>
<tr>
<td>INTROMISSION FREQUENCY</td>
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</tr>
<tr>
<td>Week 1</td>
<td>12.0 ± 4.1</td>
<td>12.25 ± 2.3</td>
<td>8.50 ± 2.5</td>
<td>20.5 ± 11.5</td>
</tr>
<tr>
<td>Week 2</td>
<td>12.8 ± 0.9</td>
<td>12.11 ± 1.3</td>
<td>13.25 ± 3.1</td>
<td>11.25 ± 1.5</td>
</tr>
<tr>
<td>Week 3</td>
<td>8.29 ± 1.0</td>
<td>11.89 ± 1.6</td>
<td>12.0 ± 4.6</td>
<td>10.0 ± 1.1</td>
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### TABLE 2 (cont.)

<table>
<thead>
<tr>
<th></th>
<th>VEHICLE</th>
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<th>STRESS-RIS</th>
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</thead>
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</tr>
<tr>
<td>Week 1</td>
<td>855.9 ± 230.7</td>
<td>690.6 ± 163.6</td>
<td>1508.6 ± 188.1</td>
<td>1722.0 ± 53.7</td>
</tr>
<tr>
<td>Week 2</td>
<td>1056.4 ± 209.6</td>
<td>531.6 ± 62.4</td>
<td>1239.0 ± 202.9</td>
<td>1549.9 ± 407.6</td>
</tr>
<tr>
<td>Week 3</td>
<td>765.6 ± 213.2</td>
<td>653.4 ± 104.9</td>
<td>1526.9 ± 156.8</td>
<td>1189.5 ± 197.9</td>
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<td><strong>POST-EJACULATORY INTERVAL</strong></td>
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<tr>
<td>Week 1</td>
<td>867.3 ± 296.6</td>
<td>603.9 ± 204.1</td>
<td>1559.7 ± 240.3</td>
<td>1800.0 ± 0.00</td>
</tr>
<tr>
<td>Week 2</td>
<td>760.8 ± 229.7</td>
<td>416.6 ± 52.4</td>
<td>1276.2 ± 321.1</td>
<td>1610.9 ± 189.1</td>
</tr>
<tr>
<td>Week 3</td>
<td>607.4 ± 298.9</td>
<td>382.6 ± 20.5</td>
<td>1535.8 ± 264.2</td>
<td>1120.8 ± 257.3</td>
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<td><strong>EJACULATION FREQUENCY</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>1.0 ± 0.4</td>
<td>1.67 ± 0.3</td>
<td>0.43 ± 0.3</td>
<td>0.20 ± 0.1</td>
</tr>
<tr>
<td>Week 2</td>
<td>1.3 ± 0.3</td>
<td>1.8 ± 0.2</td>
<td>0.71 ± 0.3</td>
<td>0.30 ± 0.2</td>
</tr>
<tr>
<td>Week 3</td>
<td>1.4 ± 0.3</td>
<td>2.0 ± 0.2</td>
<td>0.43 ± 0.2</td>
<td>0.90 ± 0.3</td>
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<tr>
<td><strong>WDS FREQUENCY</strong></td>
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</tr>
<tr>
<td>Week 1</td>
<td>2.75 ± 0.8</td>
<td>1.78 ± 0.8</td>
<td>6.43 ± 1.2</td>
<td>4.10 ± 1.2</td>
</tr>
<tr>
<td>Week 2</td>
<td>1.25 ± 0.6</td>
<td>1.22 ± 0.2</td>
<td>5.29 ± 1.2</td>
<td>5.40 ± 1.3</td>
</tr>
<tr>
<td>Week 3</td>
<td>2.38 ± 0.8</td>
<td>0.9 ± 0.4</td>
<td>7.14 ± 0.9</td>
<td>3.85 ± 0.6</td>
</tr>
</tbody>
</table>
**TABLE 3**

**WEEKLY EFFECTS OF CHRONIC METRYAPONE, CHRONIC STRESS AND CHRONIC RISPERIDONE**

**ON WDS AND SEXUAL BEHAVIOUR (MEAN ± SEM)**

<table>
<thead>
<tr>
<th></th>
<th><strong>VEHICLE</strong></th>
<th><strong>RIS</strong></th>
<th><strong>STRESS</strong></th>
<th><strong>STRESS-RIS</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MOUNT LATENCY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>985.0 ± 279.2</td>
<td>282.4 ± 263.6</td>
<td>1800.0 ± 0.0 *</td>
<td>1800.0 ± 0.0 *</td>
</tr>
<tr>
<td>Week 2</td>
<td>51.0 ± 33.3</td>
<td>270.7 ± 176.1</td>
<td>971.9 ± 281.8</td>
<td>806.9 ± 314.1</td>
</tr>
<tr>
<td><strong>MOUNT FREQUENCY</strong></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>15.6 ± 2.1</td>
<td>22.3 ± 7.4</td>
<td>—*</td>
<td>—*</td>
</tr>
<tr>
<td>Week 2</td>
<td>14.8 ± 1.9</td>
<td>24.1 ± 5.8</td>
<td>14.0 ± 4.9</td>
<td>18.2 ± 5.9</td>
</tr>
<tr>
<td><strong>INTROMISSION LATENCY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>970.1 ± 277.8</td>
<td>410.3 ± 203.9</td>
<td>1800.0 ± 0.0 *</td>
<td>1800.0 ± 0.0 *</td>
</tr>
<tr>
<td>Week 2</td>
<td>136.2 ± 58.1</td>
<td>348.4 ± 181.8</td>
<td>985.0 ± 277.1</td>
<td>820.7 ± 309.8</td>
</tr>
<tr>
<td><strong>INTROMISSION FREQUENCY</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week 1</td>
<td>14.2 ± 2.6</td>
<td>8.7 ± 1.2</td>
<td>—*</td>
<td>—*</td>
</tr>
<tr>
<td>Week 2</td>
<td>10.9 ± 2.2</td>
<td>10.9 ± 1.0</td>
<td>12.4 ± 1.0</td>
<td>11.4 ± 1.1</td>
</tr>
</tbody>
</table>

* All latencies were set to a maximum of 1800 seconds and dropped from the analyses of mount and intromission frequency when rats failed to ejaculate.
<table>
<thead>
<tr>
<th>VEHICLE</th>
<th>EJACULATION LATENCY</th>
<th>POST-EJACULATORY INTERVAL</th>
<th>EJACULATION FREQUENCY</th>
<th>WDS FREQUENCY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Week 1</td>
<td>1447.3 ± 154.1</td>
<td>1249.1 ± 269.3</td>
<td>0.6 ± 0.2</td>
<td>1.9 ± 0.8</td>
</tr>
<tr>
<td>Week 2</td>
<td>752.9 ± 127.8</td>
<td>315.9 ± 24.6</td>
<td>0.1 ± 0.2</td>
<td>1.4 ± 0.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>RIS</th>
<th>STRESS-RIS</th>
<th>STRESS</th>
<th>RIS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1800.0 ± 0.0</td>
<td>1800.0 ± 0.0</td>
<td>0.0 ± 0.0</td>
</tr>
<tr>
<td>1498.5 ± 168.2</td>
<td>1804.7 ± 228.9</td>
<td>1142.9 ± 220.6</td>
<td>0.78 ± 0.3</td>
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<tr>
<td>882.9 ± 169.4</td>
<td>1184.7 ± 228.9</td>
<td>1072.8 ± 233.9</td>
<td>1.0 ± 0.4</td>
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<tr>
<td>639.8 ± 160.3</td>
<td>198.9</td>
<td>0.8 ± 0.2</td>
<td>3.2 ± 0.7</td>
</tr>
<tr>
<td>0.8 ± 0.2</td>
<td>0.80 ± 0.3</td>
<td>3.2 ± 0.7</td>
<td>1.1 ± 0.4</td>
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<tr>
<td>0.8 ± 0.2</td>
<td>0.3</td>
<td>6.6 ± 1.9</td>
<td>1.4 ± 0.3</td>
</tr>
</tbody>
</table>

*Post-Ejaculatory Intervals were set to a maximum of 1800 seconds when rats failed to ejaculate and were dropped from analyses when rats failed to intromit following the first ejaculation.*
Weekly Effects of Chronic Stress and Risperidone on WDS Frequency in the Male Rat.

Lines Represent Means ± SEM.
Weekly Effects of Chronic Stress and Risperidone on Ejaculation Latency in the Male Rat.

Lines Represent Means ± SEM.
FIGURE 3

Weekly Effects of Chronic Metyrapone, Stress and Risperidone treatment on WDS Frequency in the Male Rat. Lines Represent Means ± SEM.