Gender Differences in Acute and Chronic Stress in Wistar Kyoto (WKY) Rats

WILLIAM P. PARÉ[1], GREG R. BLAIR,[1] JOSEPH KLUCZYNSKI,[1] AND SHANAZ TEJANI-BUTT[2]
[1]*V.A. Medical Center, Perry Point, MD*
[2]*University of the Sciences in Philadelphia, Philadelphia, PA*

Abstract—While females are considered more susceptible to depressive behavior, this assertion is not strongly supported by the experimental literature. Since stress contributes to depressive behavior, male and female Wistar Kyoto (WKY) rats were exposed to either one session (acute stress) or 5 sessions (chronic stress) of restraint plus cold in order to study depressive behavior in male and female rats. After their respective treatment exposure, rats were tested in the open field test (OFT) and for retention of a passive-avoidance (P-A) task. One stress session resulted in significant immobility in the OFT for males, whereas 5 sessions were required to produce similar immobility in female rats. Acute stress interfered with the retention of the P-A response for males, while both acute and chronic stress produced poor P-A responses in female rats. Food consumption decreased progressively, as a function of stress sessions, in female rats, whereas feeding in males returned to control levels after five stress days. Both acute and chronic stress exacerbated the stress ulcer response in male rats, but not in female rats. Chronic, but not acute, stress resulted in an increase in serotonin transporter mRNA levels in the dorsal raphe nucleus of both male and female rats. The general consensus from these data suggested that female rats were more vulnerable to chronic stress and consequently supported the notion that females may be more susceptible to stress-induced behavioral depression.
Key Words: WKY rats, acute and chronic stress, gender, passive avoidance, open field behavior, stress-ulcer, adrenal weight, serotonin, dorsal raphe nucleus

Introduction

SEX DIFFERENCES are invariably reported when male and female rats are observed in various tests of emotional behavior, such as the open field test (OFT) (Albonetti et al., 1993, Alonso et al., 1991a; Gray and Buffery, 1971; Masur et al., 1980, Meng and Drugan, 1993). Since female rats are more active in the OFT, females are judged as less emotional (Heinsbroek et al., 1988). The addition of stressful stimulation to the test situation also produces different behaviors from male and female rats with pre-shock enhancing immobility in the OFT for male rats (Kennett et al., 1986). Stress is also associated with the development of depressive behavior (Anisman and Zacharko, 1982, Willner, 1990), but in the human clinical literature, stress-related mood disorders are more prevalent in women (Dohrenwend and Dohrenwend, 1976, Weissman and Klerillan, 1977). This seemingly

Address for correspondence: William P. Paré, Ph.D., VA Medical Center, Perry Point, MD 21902. E-mail: wpare@aol.com

Integrative Physiological and Behavioral Science, October-December 1999, Vol. 34, No. 4, 227–241.

contradicts the animal data on this subject. This apparent contradiction may be due, in part, to two factors. First, the designation of depressive behavior in animal studies is frequently assigned on the basis of only one task (e.g., the Portsolt forced-swim test). Second, most animal stress studies involve an acute stressor, whereas stress-related depressive behavior may require chronic exposure to stress (Katz et al., 1981).

The present study represents an attempt to address this problem. The development of depressive behavior was studied in both male and female rats exposed to either acute or chronic stress. The behavioral impact of acute or chronic stress was evaluated in the OFT, and learning in a passive avoidance task. The peripheral physiological effects were determined from body weight changes, adrenal weight, and incidence of stomach stress ulcer.

The WKY rat strain has been proposed as an animal model for depressive behavior (Paré, 1989, Paré and Redei, 1993a). The serotonin (5–HT) system is thought to be involved in the pathophysiology of mood disorders (Hamon et al., 1990; Willner, 1985). Since the 5–HT transporters (5–HTT) are regarded, not only as initial sites of action of antidepressant drugs, but also thought to play a central role in the termination of 5–HT neurotransmission, the present study also examined the effects of acute and chronic stress on 5–HTT mRNA levels in the midbrain of male and female WKY rats.

Materials and Methods

Animals

The subjects were 26 male and 24 female 4-month old naive WKY rats raised in this laboratory from breeding stock obtained from Charles River Laboratories (Kingston, NY). Rats were housed in our animal facility with food and water *ad libitun*. Rats were individually housed two weeks before the start of the experiment. Cages were equipped with food jars that contained granular Purina Rat Chow. Daylight conditions were maintained artificially between 6 AM and 6 PM.

Apparatus

Open-field Arena. This unit was designed after the unit described by Broadhurst (1957) The open field arena was round with a diameter of 82 cm. The circular wall was 30 cm high and was constructed of aluminum sheeting. The arena was situated on a plywood floor. The floor and wall were painted with black enamel paint. The arena floor was divided by three concentric circles. The smaller inner circle had a diameter of 20 cm., the second circle had a diameter of 50 cm, and the outer circle was defined by the arena wall. Each circle was divided into essentially equal size areas. The number of areas in the inner, middle, and outer circles were 1, 6, and 12 respectively. A ceiling light was situated 132 cm above the arena floor and consisted of two fluorescent lights. Cheese cloth was draped from the ceiling and dropped outside the arena wall. The cloth served to diffuse the light and functioned as a one-way viewing screen.

Passive-avoidance apparatus. The passive avoidance (P-A) task used a light chamber-dark chamber procedure. This apparatus consisted of a Lafayette Instrument modular testing unit (Model 85000) as the dark chamber. The dark chamber was 20 x 30 x 19 cm with walls and top entry lid constructed of stainless steel. The floor consisted of stainless steel rods spaced 1.7 cm apart. The light chamber was 17 cm wide, 20 cm long, and 20 cm high. The walls, ceiling, and entry door were fabricated from 0.3 cm thick clear plastic. This

chamber also had a grid floor with stainless steel rods spaced 1.7 apart. A 52 W bulb, with reflector, was suspended 38 cm above the floor of the light chamber. The two chambers were separated by an automatic guillotine door (Model 85013, Lafayette Instruments) that provided a 9 x 9 cm opening. Opening the guillotine door activated a digital elapsed-time clock with an accuracy to 0.01 sec. Shock to the grid floor in the dark chamber was provided by a Lafayette Instrument shock generator (Model 82400) with a source voltage of 2500V/AC 60 Hz and an internal resistance of 2.4 megohms at 1 mA output.

Restraint + cold. The restraint-cold stress device consisted of 1/4–in hardware cloth tubes (diameter = 3.8 cm; length = 18 cm). Once the rat was in the tube, rods were placed through the front and the back end of the tube thereby restricting the rat inside the tube. The tube, with the rat enclosed, was placed within a ventilated refrigerator with temperature maintained at 10.5°C.

Ulcerogenic restraint device. The restraining device for the ulcerogenic procedure was fabricated from PVC tubing 23 cm long and 7.7 cm in diameter (inside dimension). The tube had four rows of 1 cm holes drilled through the length of the tube. Once the animal was in the tube, it was closed with a piece of hardware cloth and two bolts at the other end. Tubes were placed in 18.5°C water and suspended so that the water surface was level with the rat's neck.

Procedures

The different testing procedures are described herewith. The scheduling of these different procedures for the various treatment groups will be subsequently outlined.

Open Field Test (OFT). The rats were housed in single cages. The single cage, with the rat inside, was transported to the OFT room. Rats were individually placed in the inner circle. Two behaviors were recorded: latency (sec) to leave the inner circle, and the number of field segments entered with all four feet. The trial lasted 3 min after which the rat was returned to its home cage. The arena was wiped with a soap and water solution between each trial.

Passive Avoidance (P-A). P-A training and testing required three sessions, The first session served as the base trial. On this trial, rats were individually placed in the light chamber. Following a 15-sec habituation period, the guillotine door separating the light chamber from the dark chamber was opened and simultaneously started the digital clock. Once the rat entered the dark chamber, the door was closed thereby stopping the clock. The elapsed time on the clock served as the response latency measure (sec) for this test. After 30 sec in the dark chamber, the rat was returned to its home cage. The entire apparatus was cleaned with a soap and water solution between trials. The second session was identical to the first session except that entry into the dark chamber was followed by the application of a 2 sec 0.4 mA grid shock. The third session was identical to the first session and served as the test trial. If the rat failed to enter the dark chamber within 5 min, the trial was terminated.

Acute and Chronic Stress. The stress event involved immobilization plus cold exposure. Rats were immobilized in the wire mesh tubes and placed in the 10.5°C refrigerator for a 2 hr period. Stress exposure occurred between 0800 and 1200 hr. Animals assigned to the acute stress treatment received only one stress episode; animals exposed to the chronic stress treatment received 5 consecutive daily stress episodes.

Ulcerogenic Stress. Three days after the termination of their respective stress schedules, rats were exposed to the water-restraint ulcerogenic stressor. Briefly, rats were

food-deprived for 17 hr and then exposed to water-restraint for 2 hr. A 2-hr rest period followed before the rat was decapitated and the brain immediately removed and frozen at –80°C within 60 sec of decapitation. The stomach was also quickly removed and inspected for ulcers. The cumulative size of the ulcers served as the ulcer severity score.

At the same time that the stomach was removed, both adrenal glands were also removed. These were trimmed of connective and fatty tissue and a wet weight was recorded.

Determination of 5-HTT mRNA levels in brain. The midbrain region consisting of the dorsal raphe nucleus (DRN) was dissected out from the brains of the two stress groups and the Normal Control group. The dissected samples, weighing approximately 100–150 mg were placed at –80°C until the time of the assay.

RNA Isolation. Total RNA was isolated using the Trizol™ Reagent method (BRL). Briefly, the samples were homogenized in 1.5 ml of Trizol Reagent and incubated for 20 min at 15 to 30°C with periodic shaking. Chloroform (0.2 ml per ml of Trizol Reagent) was added to the samples which were shaken vigorously for 15 sec, incubated at 20°C for 10 min, and centrifuged for 15 min in a cold room. Following centrifugation, the RNA in the aqueous phase of each sample was transferred to a fresh tube. Isopropyl alcohol (0.5 ml per ml of Trizol Reagent) was added to precipitate the RNA from the aqueous phase. Samples were incubated for 3 0 min at 15–30°C, stored at –20°C for 24 hr and centrifuged 15 min in the cold room. The supernate from each tube was discarded and the RNA pellet was washed with 75% ethanol (1 ml of 75% ethanol per ml of Trizol Reagent), centrifuged, supernate discarded, and the pellet was allowed to air dry for 10 min, reconstituted and placed at –80°C for storage.

Gel Electrophoresis. 20 ug of total RNA from the treated and control brain samples was electrophoresed through denaturing 1.2% agarose/2.2M formaldehyde gel and transferred onto nylon membranes, auto cross-linked and stored in the cold room until assayed.

Hybridization. Rat 5-HTT mRNA was labeled with ^{32}P-dCTP using the random primer labeling method with the RTS (Random Primer Labeling Kit) system. Briefly, 25 to 50 ng of gel purified DNA (5-HTT or GAPDH) was added to 1X TE to a final volume of 44 ul in a small tube. The capped tube was boiled for 6 min and immediately placed on ice. The contents of the tube were added to the RTS vial, to which 6 ul of ^{32}P-dCTP was then added and mixed well by tapping the tube. The sample was incubated for 1 hr at room temperature. 5 ul of 0.2M EDTA was added to the tube to stop the reaction.

The membranes were pre-hybridized for 1 hr in 10 ml of hybridization solution (Church's Buffer) and then hybridized to the probe in 10 ml of Church's Buffer for 18 hr at 65°C. The membranes were washed to a final stringency of 2XSSC, 0.1%SDS, 0.1%SDS at 65°C. The blots were exposed to X-ray film with an intensifying screen at –70°C for 2 days for 5-HTT. For normalization of RNA, the blots were re-probed with the constitutively expressed gene GAPDH as an internal standard and exposed at-70°C for 1 hr. The blots were quantified using Brain 1.2.1 software (Drexel University).

Test Schedule for the Experimental Protocol. The experimental schedule is outlined in Figure 1. On DAY 1 all rats received one OFT trial. On the basis of the OFT behaviors, male and female rats were assigned to one of four treatment groups. These were: Acute Stress, Chronic Stress, Shock Control (i.e., no stress), and Normal Control. A Normal Control treatment was incorporated into the experimental design in order to provide a shock-free basal control group for comparison, especially for the physiological variables, with the different stress treatment conditions. Accordingly, this control group was never shocked, not even in the passive avoidance procedure. On DAY 1, all rats were exposed to the first session of the P-A procedure. On DAY 2 all rats were exposed to the second

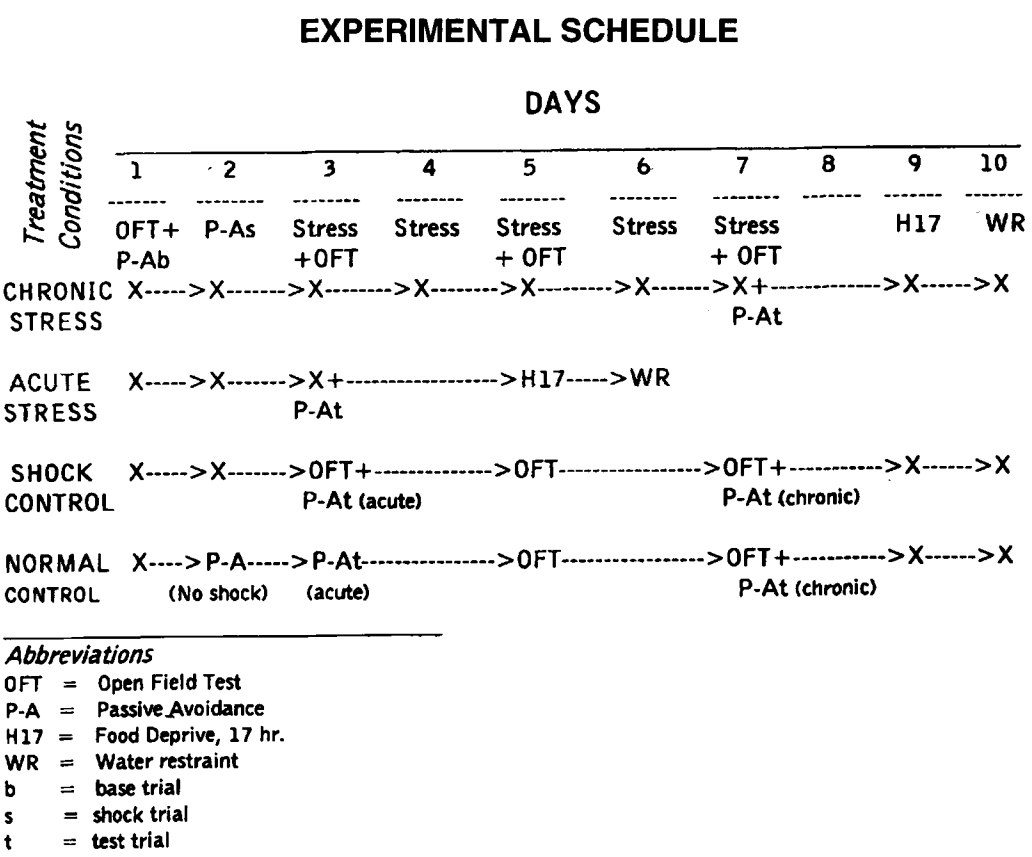

FIG. 1. Schematic outline of the experimental protocol.

session of the P-A procedure, i.e., the shock session, but Normal Control rats did not receive shock. On DAY 3 all rats, except the two control groups, were exposed to the restraint + cold stress procedure. One hr later all rats were tested in the OFT. In addition, rats in the Acute Stress Group and half of the control rats (designated the Acute Control Group) rats were exposed to the third session of the P-A procedure, which was shock free. On DAY 4 through DAY 7, Chronic Stress rats received the restraint + cold treatment daily. On DAY 5, Chronic Stress, control rats were tested in the OFT. On DAY 6 Acute Stress rats were subjected to the ulcerogenic procedure. When these rats were decapitated, the brains were frozen for subsequent 5-HTT analysis, and the stomachs were inspected for ulcers. Also on DAY 7, Chronic Stress and control rats were tested in the third, and last session, of the OFT. In addition, the Chronic Stress rats, and the other half of the Control rats (designated Chronic) were exposed to the third, test session, of the P-A procedure. On DAY 9, food was removed from Chronic Stress, and Normal Control rats at 1PM. On DAY 10 these rats were subjected to the ulcerogenic procedure.

Food consumption was measured daily throughout the experimental period. The unit of analysis consisted of the percentage difference in daily food consumption of Acute Stress and Chronic Stress animals as compared to the food consumed by Normal Control rats for that particular day.

Statistical Analysis. The data were analyzed using a two-factor ANOVA design with

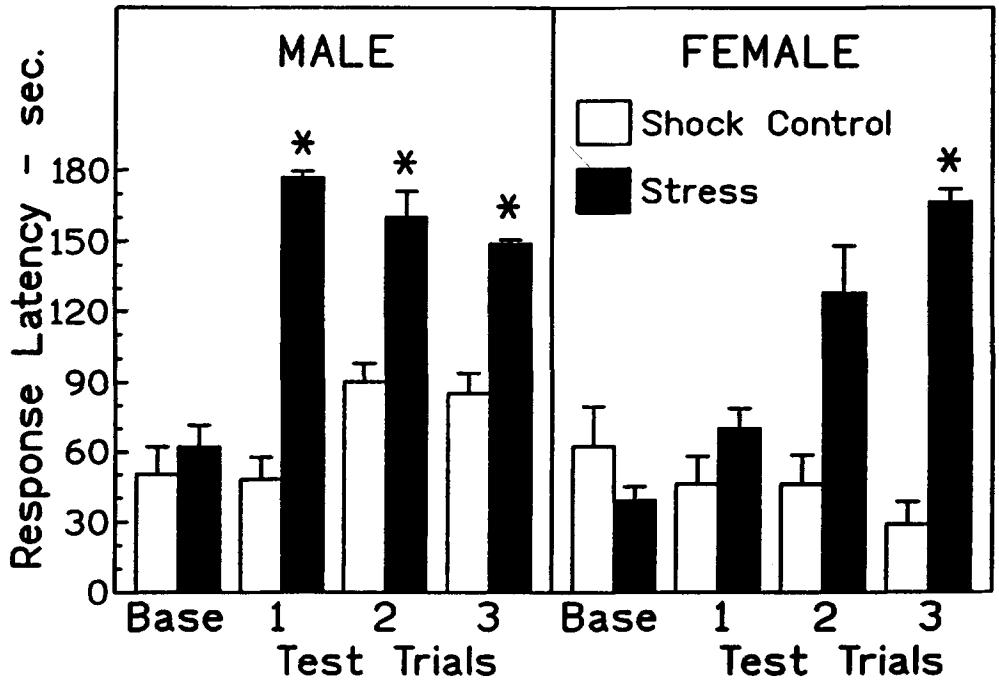

FIG. 2. Mean (±SE) response latency to leave the start section in the open-field test for male and female rats, for the pre-stress base trial and the three test trials. Test trials 1, 2 and 3 occurred after 1, 3 and 5 days, respectively, of restraint+cold stress. *$p<0.05$ significant difference from the appropriate Shock Control group for the same test session.

SEX as one factor (2 levels) and TREATMENT as the other factor (4 levels). The food consumption data were analyzed using a two-factor ANOVA design with SEX (2 levels) contributing the first factor and the second factor consisting of the 8 repeated daily feeding measures. The OFT data were analyzed using a three-factor ANOVA, with SEX as one factor, TREATMENT as the second factor (Chronic Stress versus Normal Controls), and TRIALS (with four levels) contributing the repeated measure. The Tukey honestly significant difference (HSD) test with a p value of 0.05 was applied only to locate significant differences between groups to address specific experimental questions.

Results

Open Field Test (OFT)

Since there was no differences between the OFT data on Test Day I for both Acute Stress and Chronic Stress rats, the data from the Chronic Stress rats was used to evaluate both acute and chronic stress effects. Analysis of the response latency data revealed that latency scores were significantly greater for male rats, $F(1, 21) = 6.00$, $p<0.01$, and that the stress procedure was associated with a significant increase in latency scores, $F(1, 29) = 102.80$, $p<0.01$, as compared to the Normal Control procedure. Response latency also increased significantly during the test trials, $F(3, 63) = 11.20$, $p<0.01$, as compared to the base trials. The significant triple interaction term, $F(33, 63) = 3.65$, $p<0.05$, which allowed

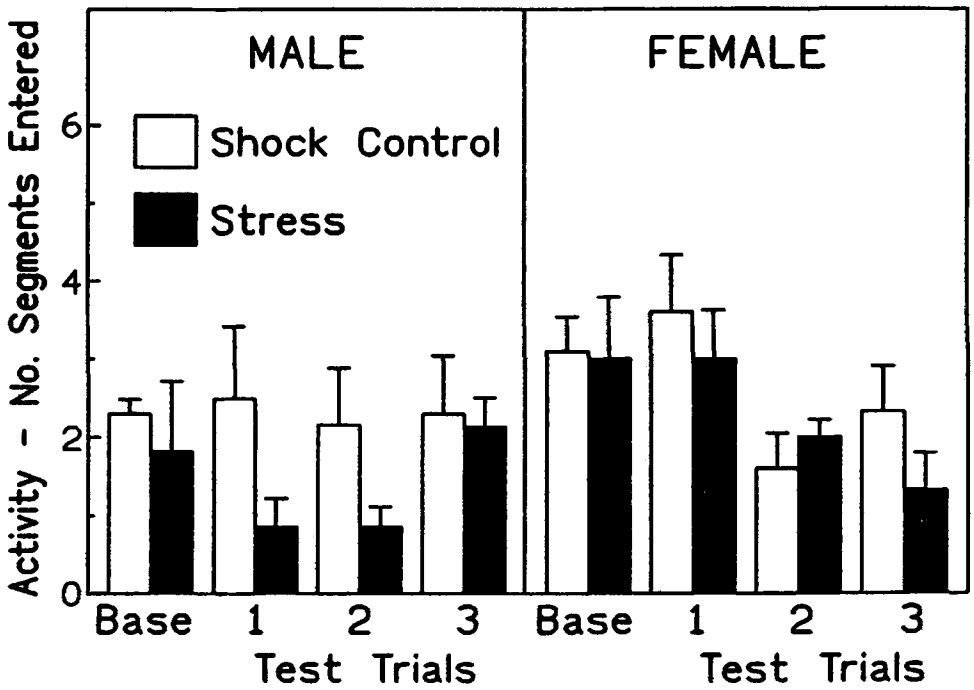

FIG. 3. Mean (±SE) number of segments crossed (i.e., the motor activity measure) for the prestress base trial and the three test trials and in the open-field test for male and female rats.

for the comparison between individual treatment means, showed that the stress schedule resulted in a significant increase in response latency scores for male rats after the first stress episode, whereas a similar increase did not materialize for the female rats until after the third OFT test session. These data are illustrated in Figure 2.

The measure of activity within the OFT, namely "Number of Segments Entered," indicated that female rats were more active than male rats, $F(1, 21) = 4.68$, $p<0.05$. Although group means suggested that, as a function of test trials, male stressed rats became more active and female stressed rats became less active, these differences were not significantly different. These data are illustrated by Figure 3.

Passive Avoidance

These data were analyzed to determine if either acute or chronic stress had a differential effect, associated with sex, on the retention of a passive avoidance response. In the original design, Acute Stress rats were compared to Acute Control rats and Chronic Stress rats compared to Chronic Control rats. However, there were no significant differences between the two Control subgroups. Therefore, these data were pooled in order to simplify comparisons between treatment conditions. Rats exposed to the stress treatments did not retain the P-A response as well as Control rats, $F(3,46) = 107.49$, $p<0.01$. On the test day, male rats acquired the passive avoidance response more readily (i.e., had higher mean response latency scores) as compared to female rats, $F(1,46)$ 12.39, $p<0.01$. There were significant differences between treatment conditions, $F(3,46)$ 2.92, $p<0.05$. Acute Stress, but not

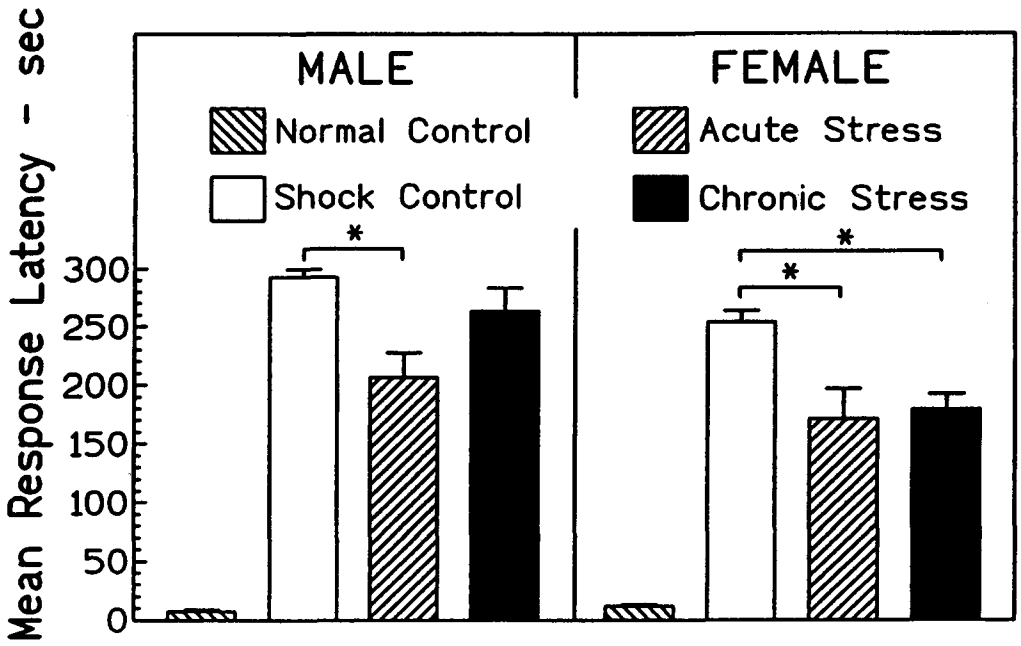

FIG. 4. Mean (±SE) response latency on the test trial in the one-way passive avoidance task. High scores reflect acquisition and retention of the P-A response. A trial was terminated after 300 sec. *$p<0.05$.

Chronic Stress, diminished the magnitude of the P-A response in male rats, but as Figure 4 shows, the magnitude of the P-A response in female rats was significantly diminished by both the Acute and the Chronic Stress treatments, Tukey HSD test, $p<0.05$.

Daily Food Consumption

The feeding data were analyzed only for the chronic stress treatment animals since it was not anticipated that any acute stress effects could be observed on the first stress day of acutely stressed rats. Accordingly, male and female chronically stressed rats were compared. Exposure to stress resulted in a decrease in daily food consumption, $F(7, 77) = 6.72$, $p<0.01$, but while diminished feeding persisted with female rats, male feeding returned to baseline and was significantly greater than female feeding on the last data collection day (Days X Sex interactions, $F(7,77) = 3.96$, $p<0.01$). These data are illustrated by Figure 5.

Adrenal Weight/Body Weight Ratio

Adrenal glands were significantly heavier in female rats, as compared to male rats, $F(1, 42) = 225.35$, $p<0.01$, and as Figure 6 indicates, the chronic stress schedule produced larger adrenal ratios for both male and female rats, but these differences were not significant.

Stress Ulcer

Ulcer severity scores were significantly greater in male rats, $F(1, 42) = 65.73$, $p<0.01$.

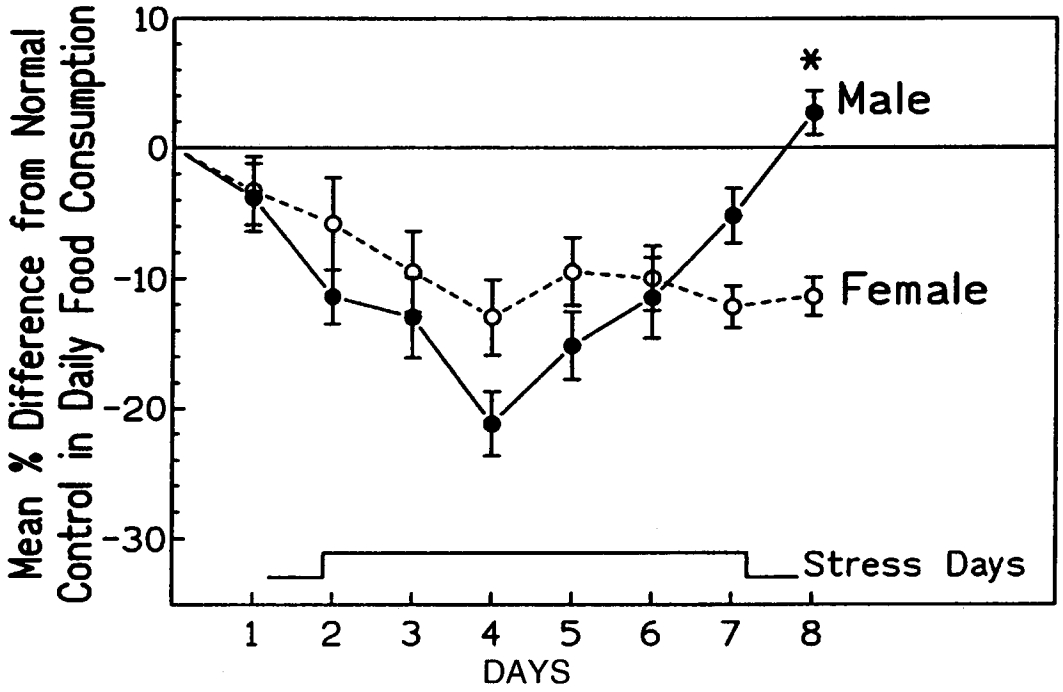

FIG. 5. Mean (±SE) percent difference in feeding for both male and female rats as compared to their respective sex normal control rats. Stress Days include the passive avoidance shock day. *$p<0.05$ for the difference between male and female rats.

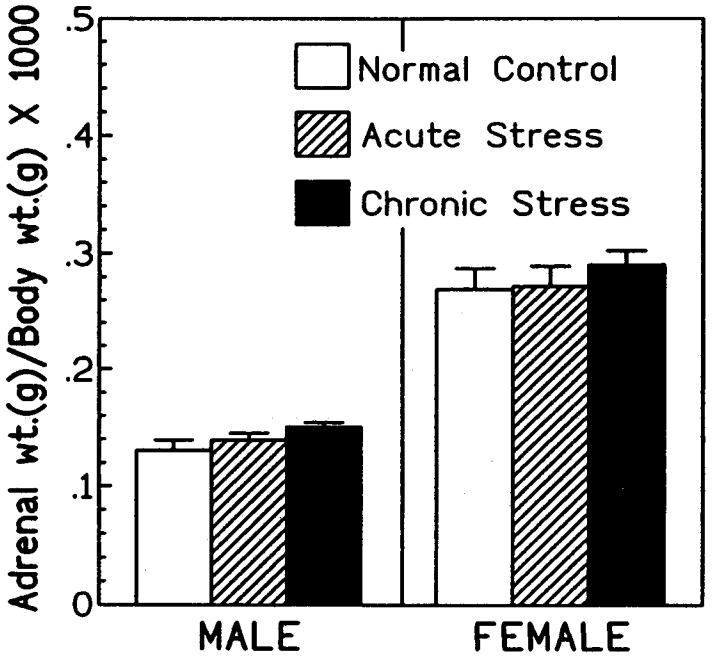

FIG. 6. Mean (±SE) adrenal weight (g)/ body weight (g) X 1000 ratios for male and female rats exposed to either Acute Stress, Chronic Stress, or the Normal Control treatments.

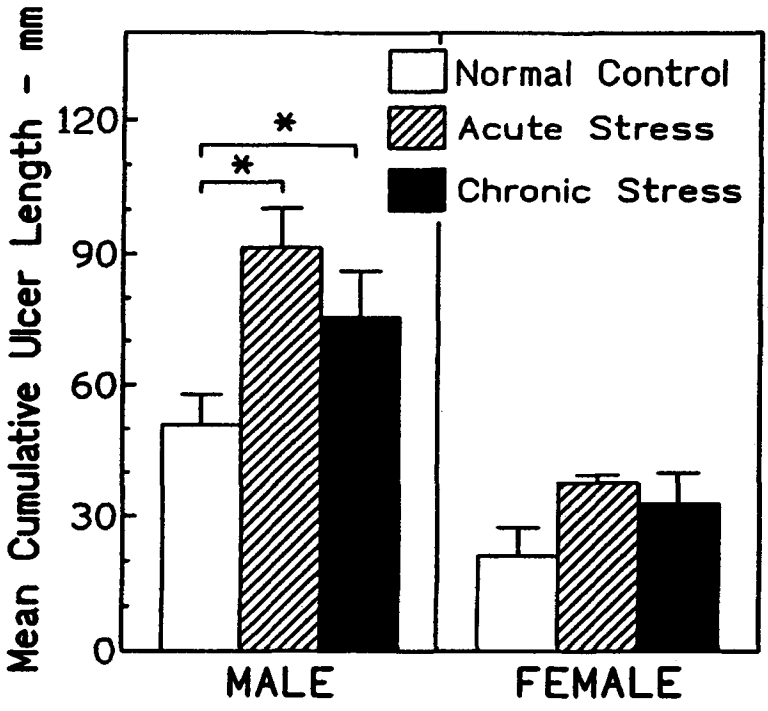

FIG. 7. Mean (±SE) cumulative ulcer length (mm) for male and female rats exposed to either the Normal Control, Acute Stress, or the Chronic Stress treatments. *$p<0.05$, significant group differences between the control group as compared to both the acute stress and the chronic stress groups.

As Figure 7 suggests, prior stress exposure facilitated subsequent ulcer development. However, this effect was observed only with male rats, Tukey HSD test, $p<0.05$.

5-HTT mRNA levels

Male rats had significantly higher 5-HTT values as compared to female rats, $F(1,30) = 12.39$, $p<0.05$. There was a significant Treatment effect, $F(2,30) = 8.80$, $p<0.01$, which was attributable to the higher 5-HTT values for rats exposed to the Chronic Stress treatment as compared to the Normal Control rats, Tukey HSD test, $p<0.05$. This effect was not gender specific. Acute stress had no effect of 5-HTT mRNA sites in either male or female rats. However, 5-HTT mRNA levels was significantly lower in female Normal Control rats as compared to male Normal Control rats, Tukey HSD test, $p<0.05$. These data are shown in Figure 8.

Discussion

Male and female rats responded differently to stress. The majority of the data suggested that male rats were more responsive to acute stress and exhibited some form of adaptation when exposed to repeated stress, whereas female rats were less responsive to acute stress but more vulnerable to chronic stress. While the majority of the data would suggest such a response pattern between the sexes, some of the variables observed in this study did not conform to this conclusion.

The OFT data were in agreement with prior studies which reported that female rats are

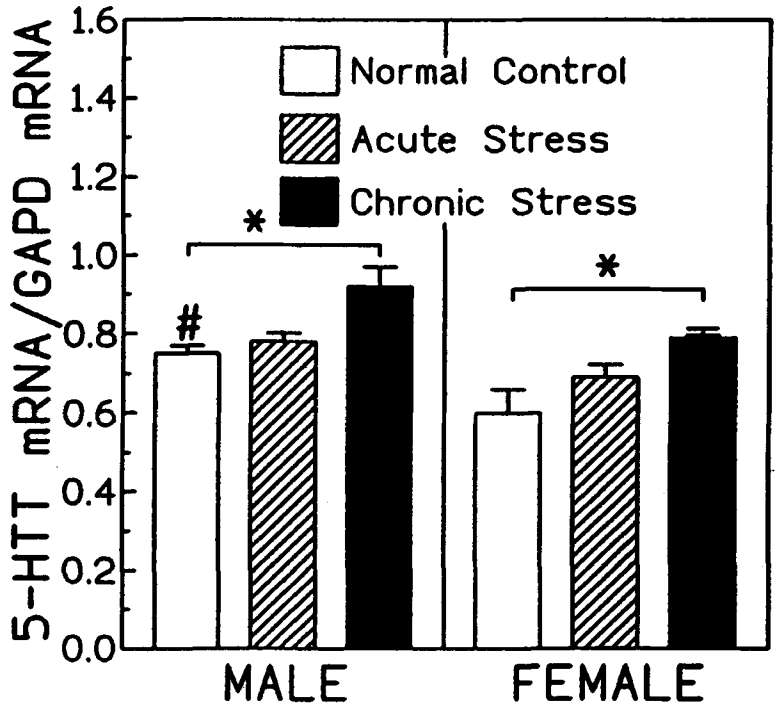

FIG. 8. Mean (±SE) 5-HTT mRNA/GAPD levels in the midbrain of male and female rats. 5-HTT values were significantly greater in chronically stressed male and female rats as compared to their respective Normal Control rats, *$p<0.05$. #Male Normal Control levels significantly greater than female Normal Control levels, $p<0.05$.

more active in the OFT (Alonso et al., 1991b; Archer, 1975; Guilamin et al., 1990; Mazur et al., 1978; Meng and Drugan, 1993) and that reduced activity in the OFT as a result of prior shock was greater in male rats (Heinsbroek et al., 1988). The OFT data suggested that female rats were less responsive to acute stress and become less mobile when exposed to repeated shock sessions. These data are in accord with Kennett's report which showed that females were less affected by a single stress session but failed to adapt to repeated stress exposures (Kennett et al., 1986).

Intense stress inhibits food intake (Kennett et al., 1986; Krahn et al., 1986; Paré, 1965; Sterritt and Shemberg, 1963) with the consequent outcome of a reduction in body weight (Armario et al., 1995; Ottenweller et al., 1989, 1992, Paré, 1964). In the present study, chronic stress was associated with a differential decrease in daily food consumption for male and female rats. While the initial decrease in feeding for males was greater than that observed in females, daily feeding for males returned to control levels whereas females never regained pre-stress levels, As previously reported, this difference in feeding, may reflects a gender response difference in the feeding pattern to stress (Brown and Grunberg, 1996).

The passive avoidance data also suggested that chronic stress had a greater impact on female rats. One episode of cold-restraint stress interfered with the elaboration of the passive avoidance response in both male and female rats. But this effect diminished with male rats whereas it persisted in female rats, suggesting that chronic stress had greater

effect on females. However, female rats may be biased in this response since female rats, under non-stress conditions, are more prone to enter a dark compartment (Heinbroek, et al. 1988). Consequently, on P-A test trials, this dark chamber preference by females may have interfered with the avoidance behavior of staying in the illuminated chamber.

Adrenal hypertrophy is associated with chronic stress (Armerio et al., 1990; Pare, 1971), and this hypertrophy is more pronounced in female rats (Baldwin et al., 1997), which reflects the greater sympathoadrenal system response to stress in female rats (Weinstock et al., 1998). Since the adrenal hypertrophy mirrors the cellular changes in the adrenal cortex, the brief stress resulting from the acute stress condition may not have represented a sufficient stimulus condition to produce a stress-related hypertrophy. In addition, this physiological response may not be sufficiently sensitive to detect sex differences in adrenal function, if indeed these exist.

The ulcer data agree with previous reports showing that prior exposure to uncontrollable shock stress exacerbated ulcers later induced by water-restraint stress (Glavin et al., 1991; Murison and Overmier, 1986, Overmier and Murison, 1988). However, the greater incidence of ulcers in male rats, as compared to female rats, was not in accord with other reports (Herner and Caul, 1971; Paré and Redei, 1993b). This discrepancy may reflect the lack of experimental control for the estrus phase in female rats in the present study. Due to the length of the experimental protocol, the present study did not control for estrus phase. The fact that female rats during diestrous, as compared to proestrus-estrus, are less susceptible to stress ulcer (Paré and Redei, 1993b) suggests the possibility that the majority of female rats in the present study may have been in diestrous during the ulcerogenic procedure.

Serotonin transporter (5-HTT) are derived from a large family of monoamine transporters and play a central role in the termination of 5-HT neurotransmission via uptake into presynaptic neurons. 5-HTT sites are also regarded as initial sites of antidepressant drugs (Marcusson and Ross, 1990). Previous studies have hypothesized that mRNA transcription may be important for the regulation of 5-HTT function, and that 5-HTT gene regulation may be involved in the long term adaptative changes that are seen following repeated antidepressant treatments (Lesch et al., 1993; Lopez et al., 1994). In the present study, we examined whether a single exposure, or repeated exposure to stress would alter 5-HTT mRNA levels in the brains of male and female WKY rats. The cloning of cDNA for 5-HTT (Blakely et al., 1991; Hoffman et al., 1991) has made it possible to study the expression levels of 5-HTT in the most highly expressed brain region, namely, the dorsal raphe and median raphe nuclei located in the midbrain region.

It has been suggested that a decrease in 5-HT neurotransmission may be responsible for depressive behavior (Yavari et al., 1993). The literature also suggests that stressors that do not allow for adaptation (Watanabe et al., 1993) produce behavioral depression which is accompanied by a decrease in 5-HT activity. Recent clinical data indicate significant male and female differences in 5-HT synthesis rate suggesting that sexual differences in the 5-HT system may contribute to the pathophysiology of some psychiatric conditions (Nishizawa et al., 1997). Preclinical studies have shown that female rats tend to have a higher rate of 5-HT synthesis than male rats (Carlson and Carlson, 1988a, 1988b; Haleem et al., 1990, Alonso et al., 1991b), and demonstrate less immobility in the Porsolt swim test than male rats (Alonso et al., 1991a).

We have reported that WKY rats do not readily adapt to stress as indicated by behavioral, endocrine and neuroendocrine measurements (Paré and Redei, 1993a; Tejani-Butt et

al., 1994, Paré and Tejani-Butt, 1996; Zafar et al., 1997). In addition, 5-HTT binding sites were increased in the midbrain region of WKY but not Sprague Dawley rats that were subjected to 21 days of novel stressors (Paré and Tejani-Butt, 1996).

The present study support our previous observations that WKY rats are hyperresponsive to stress and provides new information regarding gender differences in their stress response. Our behavioral data revealed that, while male WKY rats demonstrated significant immobility in the OFT following a single stress session, 5 stress sessions were necessary to produce similar immobility measures in female rats. This differential gender effect supports previous reports regarding gender differences to acute and chronic stress (Kennett et al., 1986).

An analysis of the midbrain region indicated that control female WKY rats had significantly lower 5-HTT mRNA levels than their male counterparts. A decrease in 5-HTT gene expression may be related to a decrease in 5-HTT binding sites, leading to an increase in 5-HT levels in the synaptic cleft. This noted gender difference in 5-HTT mRNA levels in non-stressed WKY rats may be responsible for the behavioral differences observed in the present study.

A single stress session did not alter 5-HTT mRNA levels in either male or female rats. However, repeated stress exposure led to a significant increase in 5-HTT mRNA levels in both male and female rats compared to non-stressed controls. Thus it is tempting to speculate that an increase in 5-HTT mRNA activity following repeated stress could lead to an increase in 5-HTT binding capacity, resulting in a decrease in synaptic 5-HTT levels. A blunted response to the HPA axis in the WKY rat strain may lead to a persistent decrease in 5-HT activity, thereby perpetuating the behavioral depression which may be linked to decreased 5-HT activity (Redei et al., 1994; Paré and Tejani-Butt, 1996). Alternately, since WKY rats do not readily adapt to stress as compared to other rat strains, an increase in 5-HTT mRNA levels in female rats following repeated episodes of the same stressor may indicate an adaptive response aimed at maintaining homeostatic levels of 5-HT in the synaptic cleft.

At the present time, it is not clear why the effect of stress appear to be more striking in female than in male rats. Sex differences in various behavioral paradigms have been previously reported. While female rats, as compared to male rats, are reported to be less vulnerable to the effects of acute stress (Gray and Buffery, 1971; Kennett et al., 1986; Masur et al., 1980) , we have observed that female WKY rats are more vulnerable to chronic stress (Paré and Redei, 1993) and this would be in agreement with clinical observations that sex related mood disorders as well as a decrease in coping mechanism are more prevalent in women than in men (Weissman and Klerman, 1977).

These observations, when taken together (i.e., a blunted response to corticosterone or a dysfunctional HPA axis, along with the influence of sex steroids), suggest that the steroid hormonal milieu in this "depressed" rat strain may be playing a determining role on both the behavioral as well as neurochemical changes observed in the present study. However, since the present study did not measure 5-HT levels, nor control for estrus cycle effects on stress reactivity and 5-HTT mRNA levels, these observations provide for interesting speculation that require further investigation.

In conclusion, the results of the present study suggest that female rats were less responsive to acute stress stimulation when compare to male counterparts, and that 5-HTT gene expression may be more susceptible to stress induced alterations in female rats as compared to male rats.

Acknowledgments

This research was supported from Medical Research Service funds from the Department of Veterans Affairs (W.P.P.).

References

Albonetti, M.E. and Farabollini, F. (1993) Effects of single and repeated restraint on the social behavior of mate rats. *Physiology and Behavior*, 53: 937–942.

Alonso, S.J., Arevalo, R., Afonso, D., and Rodriguez, M, (1991). Effects of maternal stress during pregnancy on forced swimming test behavior of the offspring. *Physiology and Behavior*, 50: 511–517.

Alonso, S.J., Castellano, M.A., Afonso, D., and 1 Rodriguez, M. (1991). Sex differences in behavioral despair: Relationships between behavioral despair and open field activity. *Physiology and Behavior*, 49: 69–72.

Anisman, H and Zacharko, R.M. (1982). Depression: The predisposing influence of stress, *Behavioral Brain Research*, 5: 89–137.

Archer, J. (1975). Rodent sex differences in emotional and related behavior. *Behavioral Biology*, 14: 451–479.

Armario, A., Marti, J. and Gil, M (1990). The serum glucose response to acute stress is sensitive to the intensity of the stressor and to habituation. *Psychoneuroendocrinolgy*. 15: 341–347.

Baldwin, D.R., Wilcox, Z.C., and Zheng, G. (1997). The effects of voluntary exercise and immobilization on humoral immunity and endocrine responses in rats. *Physiology and Behavior*, 61: 447–453.

Blakely, R.D., Berson, H.E., Fremeau, Jr., M.G., Caron, M.M., and Bradley, C.C. (1991). Cloning and expression of functional serotonin transporter from rat brain. *Nature*, 354: 66–70.

Broadhurst, P.L. (1957). Determinants of emotionality in the rat. 1. Situational factors. *British Journal of Psychology*, 48:1–12.

Brown, K.L. and Grunberg, N.E. (1996). Effects of environmental conditions on food consumption in female and male rats. *Physiology and Behavior*, 60: 293–297.

Carlsson, M. and Carlsson, A. (1988a). A regional study of sex differences in rat brain serotonin. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 12: 53–61.

Carlsson, M. and Carlsson, A. (1988b). In vivo evidence for a greater brain tryptophan hydroxylase capacity in female than in male rats. *Naunyn-Schmiedbergs Archives Pharmacology*, 338: 345–349.

Dohrenwend, B.P. and Dohrenwend, B.S.(1976). Sex differences and psychiatric disorders. *American Journal of Sociology*, 81: 1447–1454.

Glavin, G.P., Murrison, R., Overmier, J.B., Paré, W.P., Bakke, P.G., Henke, P.G., and Hernandez, D.E. (1991). The neurobiology of stress ulcer. *Brain Research Reviews*, 16: 301–343.

Gray, J.A. and Buffery, A.W.H. (1971). Sex differences in emotional and cognitive behavior in mammals including man: Adaptive and neural basis. *Acta Psychologica*, 35: 89–111.

Guillamon, A., Cales, J.M. Rodriguez-Zafra, M., Perez-Laso, C., Caminero, A., Izquierdo, M.A.P., and Segovia, S (1990). Effects of prenatal diazepam administration on two sexually dimorphic nonreproductive behaviors. *Brain Research Bulletin*, 25:913–916.

Haleem, D., Kennett, G., and Curzon, G. (1990). Hippocampal 5-HT synthesis is greater in female rats than in males and more decreased by 5-HT1A agonist 8-OH-DPAT. *Journal of Neural Transmission*, 79: 93–101.

Hamon, M., Gozlan., H., Mestikawy, S.E., Emerit, M.B., Bolanos, F., and Schechter, L. (1990). The central 5-HT1A receptors: Pharmacological, biochemical, functional and regulatory properties. *Annals of the New York Academy of Science*, 600: 114–131.

Heinsbroek, R.O.W., van Haaren, F., and van de Poll, N.E. (1988). Sex differences in passive avoidance behavior of rats: Sex-dependent susceptibility to shock-induced behavioral depression. *Physiology and Behavior*, 43: 201–206.

Hoffman, B.J., Mezey, E., and Browstein, M.J. (1991). Cloning of a serotonin transporter affected by antidepressants. *Science*, 254: 579–580.

Katz, R.J., Roth, K.A., and Carroll, B.J. (1981). Acute and chronic stress effects on open field activity in the rat: Implications for a model of depression. *Neuroscience and Biobehavioral Reviews*, 5: 247–251.

Kennett, G.A., Caouloff, F., Marcou, M., and Curzon, G. (1986) Female rats are more vulnerable than males in an animal model of depression: The possible role of serotonin. *Brain Research*, 382: 416–421.

Krahn, D.D., Gosnell, B.A., Grace, M., and Levine, A.S.(1986). CRF antagonist partially reverses CRF-stress-induced effects on feeding. *Brain Research Bulletin*, 17: 285–289.

Lesch, P.K., Aulakh, C.S., Wolozin, B.L., Tolliver, T.J., Hill, J.L., and Murphy, D.L. (1993). Regional brain

expression of serotonin transporter mRNA and its regulation by reuptake inhibiting antidepressants. *Molecular Brain Research*, 17, 31–35.

Lopez, J.GF., Chalmers, D. T., Vazquez, D. M., Watson, S. J., and Akil, H.(1994). Serotonin transporter mRNA in rat brain is regulated by classical antidepressants. *Biological Psychiatry*, 35, 287–290.

Marcusson, J.O., and Ross, S.B.(1990). Binding of some antidepressants to the 5-Hydroxytryptamine transporter in brain and platelets. *Psychopharmacology*, 102, 145–155.

Mazur, J., Schutz, M.T. and Boerngen, R. (1978). Gender differences in open-field behavior as a function of age. *Developmental Psychobiology*, 13:107–110.

Meng, I.D. and Drugan, R.C. (1993). Sex differences in open-field behavior in response to the carboline FG 7142 in rats. *Physiology and Behavior*, 54: 701–705.

Nishizawa, S., Benkelfat, C., Young, S., Leyton, M., Mzengeza, S., de Montigny, C., Blier, P., and Diksic, M. (1997). Differences between males and females in rates of serotonin synthesis in human brain. *Proceedings of the National Academy of Science*, 94: 5308–5313.

Ottenweller, J.E., Natelson, B.H., Pitman, D.L., and Drastal, S.D. (1989). Adrenocortical and behavioral responses to repeated stressors: Toward an animal model of chronic stress and stress-related mental illness. *Biological Psychiatry*, 26: 829–841.

Ottenweller, J.E., Servatius, R.J., Tapp. W.P. Drastal, S.D., Bergen, M.T., and Natelson, B.H. (1992). A chronic stress state in rats: Effects of repeated stress on basal corticosterone and behavior. *Physiology and Behavior*, 51: 089–698.

Paré, W.P. (1964). The effects of chronic environmental stress on stomach ulceration, adrenal function, and consummatory behavior in the rat. *Journal of Psychology*, 57: 143–151.

Paré, W.P. (1965), Stress and consummatory behavior in the albino rat. *Psychological Reports*, 16: 399–405.

Paré, W.P. (1971). Frequency and probability of US-shock and adrenal hypertrophy in the rat. *Physiology and Behavior*, 6: 493–496.

Paré, W.P. (1989). Stress ulcer susceptibility and depression in Wistar Kyoto (WKY) rats. *Physiology and Behavior*, 46:993–998.

Paré, W.P. and Glavin, G.B. (1986). Restraint stress in biomedical research: A review. *Neuroscience and Biobehavioral Review*, 10: 381–370.

Paré, W.P. and Tejani-Butt, S.M. (1996). Effect of stress on the behavior and 5-HTT system in Sprague Dawley and Wistar Kyoto rat strains. *Integrative Physiological and Behavioral Science*, 31: 112–121.

Paré, W.P. and Redei, E. (1993a). Depressive behavior and stress ulcer in Wistar Kyoto rats. *Journal of Physiology* (Paris), 87: 229–238.

Paré, W.P. and Redei, E. (1993b). Sex differences and stress response of WKY rats. *Physiology and Behavior*, 54: 1179–1185.

Redei, E., Paré, W.P., Aird, F., and Kluezynski, J. (1994). Stress differences in hypothalamic-pituitary-adrenal activity and stress ulcer. *American Journal of Physiology*, 266: R353–R360.

Sterritt, G.M. and Shemberg, K. (1963). Inhibition and facilitation of eating by electric shock. II. Shock level, shock schedule and strain of rats. *Journal of Psychosomatic Research*, 7: 215–222.

Tejani-Butt, S.M., Paré. W.P., and Yang, J. (1994). Effect of repeated novel stressors on depressive behavior and brain noradrenergic system in Sprague Dawley and Wistar Kyoto rats. *Brain Research*, 649: 27–35.

Watanabe, Y., Sakai, R.R., McEwen, B.S., and Mendelson, S. (1993). Stress and antidepressant effects on hippocampal and cortical 5-HT2 receptors and transport sites for serotonin. *Brain Research*, 615: 87–94.

Weissman, M.M., and Klerman, G.L. (1977). Sex differences and the epidemiology of depression. *Archives of General Psychiatry*, 34: 98–111.

Weinstock, M., Razin, M., Schorer-Apelbaum, D., Men, D., and McCarty, R. (1998). Gender differences in sympathoadrenal activity in rats at rest and in response to footshock stress. *International Journal of Developmental Neuroscience*, 16: 289–295.

Willner, P. (1990). Animal models of depression: An overview. *Pharmacology and Therapeutics*, 45: 425–455.

Willner, P. (1985). Antidepressants and serotonergic neurotransmission: An integrative review. *Psychopharmacology*, 85: 387–404.

Yavari, P., Vogel, G.W., and Neill, D.B. (1993). Decreased raphe unit activity in a rat model of endogenous depression. *Brain Research*, 611: 31–36.

Zafar, H.M., Paré, W.P., and Tejani-Butt, S.M. (1997). Effects of acute or repeated restraint stress on behavior and brain norepinephrine system in Wistar Kyoto rats. *Brain Research Bulletin*, 44: 289–295.