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Acute stress impairs spatial memory in male but not female rats: influence of estrous cycle

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Abstract

We investigated how sex and estrous cycle influenced spatial recognition memory in the Y-maze after exposure to acute restraint stress. In Experiment 1, intact male and female rats were restrained for 1 h and then 2 h after the start of restraint, rats were trained on the Y-maze. After a 4 h delay, hippocampal-dependent spatial recognition memory was assessed. Acute stress produced opposite patterns between the sexes with spatial memory being impaired in males and facilitated in females. Serum corticosterone measures indicated that both sexes showed a robust corticosterone response after restraint and a moderate corticosterone response after Y-maze exposure. Serum corticosterone levels in response to restraint and Y-maze were not statistically different between the sexes. Experiment 2 examined the influence of the estrous cycle on spatial memory ability after acute stress. Acute stress facilitated spatial memory in females compared to controls, regardless of the estrous cycle phase (estrus and proestrus). Moreover, females in proestrus showed higher serum corticosterone levels during restraint compared to females in estrus. No differences in corticosterone levels were observed at baseline or following 2 h of recovery from restraint. These data show important differences in how sex and estrous cycle influence cognitive functions following acute stress.

Keywords: Spatial memory; Acute stress; Female; Estrus; Proestrus; Sex difference

1. Introduction

Exposure to both chronic and acute stress has substantial effects on learning and memory (for reviews, Cahill and McGaugh, 1998; Kim and Diamond, 2002; LeDoux, 2000; McEwen and Sapolsky, 1995). Chronic stress generally produces deficits on hippocampal-dependent memory processes in male rats (Conrad et al., 1996; Luine et al., 1994; Park et al., 2001), while enhancing these processes in females (Bowman et al., 2001, 2002, 2003; Conrad et al., 2003). The deficits observed in males may be associated with chronic stress-induced restructuring of the hippocampus (Conrad et al., 1999b; Magariños and McEwen, 1995; Sousa et al., 2000; Watanabe et al., 1992), which is not extensive in females (Galea et al., 1997). The effects of acute stress on learning and memory are less consistent. In male rats, acute stress enhances memory performance on highly aversive tasks (Blank et al., 2002; Sananbenesi et al., 2003; Shors, 2001; Shors et al., 1992), impairs memory performance on appetitive spatial navigation tasks (Diamond et al., 1996; Shors and Dryver, 1992) and object recognition tasks (Baker and Kim, 2002; Beck and Luine, 2002), or has no effect (Warren et al., 1991; Youngblood et al., 1997). There are many differences across these experiments that might contribute to the diversity of outcomes. These include not only appetitive versus aversive components of the task, but differences in the type of stressor, task difficulty (Diamond et al., 1999), and timing between stress and training (Blank et al., 2002). In addition, acute stress may influence cognitive search strategies, such as cue utilization (Kim and Baxter, 2001). For example, acute restraint and tailshock stress just prior to training biases male rats to use hippocampus-insensitive tactics, such as cued or stimulus–response (S–R) learning (Kim et al., 2001). Moreover, disruption of the amygdala interferes with this stress-induced shift, but leaves the response of

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the hypothalamic–pituitary–adrenal axis intact (Maroun and Richter-Levin, 2003), suggesting that stress may modulate the interaction between memory systems through amygdala activation and synaptic plasticity (for review, see Conrad, in press).

Sexual dimorphisms in the stress response may be another important contributor to the differential effects of acute stress on learning and memory. Many examples of sexual dimorphisms in the stress response have been reported, including differences in adrenocorticotropin levels (Handa et al., 1994), corticosterone levels (Critchlow et al., 1963; Galea et al., 1997; Haleem et al., 1988; Kant et al., 1983; Kitay, 1961), corticosterone binding in the brain (MacLusky et al., 1996; Turner, 1992; Turner and Weaver, 1985), and hippocampal activation (Figueiredo et al., 2002). A growing literature in humans and rodents suggests that acute stress also produces sex differences in cognition (Shors et al., 2000; Wolf et al., 2001). Shors et al. have shown that acute exposure to restraint and tail shock stress in rats enhances eyeblink delay conditioning in males, but impairs performance in females (Wood and Shors, 1998). However, the literature is limited on the influence of acute stress on learning and memory processes using females.

The different effects of stress on memory between male and female rats might reflect the influence of the ovarian steroids across the estrous cycle. Circulating ovarian steroids may predispose females to the impairing action of acute stress on certain tasks because ovariectomy prior to stress attenuates the impairment (Wood et al., 2001). Furthermore, rats at proestrus, when gonadal steroids are high, show significantly fewer conditioned responses following acute stress than rats at estrus and diestrus, when gonadal steroids are low or rising (Shors et al., 1998). Additionally, chronically stressed rats at proestrus show impaired acquisition on the radial arm maze, while rats in estrus and diestrus are unimpaired on acquisition (Bowman et al., 2001). In related work, stress or arousal during performance of a variety of tasks may interact with ovarian hormone status to produce learning and memory deficits when estrogen is high, unless habituation to the task has occurred (Frye, 1995; Markus and Zecevic, 1997; Perrot-Sinal et al., 1996). Perhaps, acute stress alters memory ability through enhancing the levels of estradiol (Shors et al., 1999). Together, the results suggest that responses to acute stress exposure are sexually dimorphic and that cyclic changes in circulating ovarian steroids across the estrous cycle will produce different outcomes, with proestrus (high estrogen) impairing performance and estrus (low estrogen) facilitating performance following acute stress.

While acute stress may interact with sex and estrous cycle to define the magnitude and direction of memory effects, demonstrations have included only a few tasks, including the radial arm maze, T-maze, water maze, and Pavolvian conditioning. Determining the effects of acute stress on other tasks is necessary to better characterize the role of sex and related gonadal hormones. This need is especially evident, given that increases in circulating estrogen in nonstressed animals facilitate hippocampal-dependent learning and memory, but are ineffective or impair performance on other tasks, such as those requiring egocentric or cued strategies (Korol and Kolo, 2002; for review, see Dohanich, 2002). Thus, estrous cycle and ovarian hormones may produce different outcomes on hippocampal-dependent tasks between control and stressed females.

The present experiments assess the effects of acute stress on memory for a spatial recognition task previously shown to be sensitive to hippocampal manipulations (Conrad et al., 1996). Experiment 1 tested the hypothesis that acute restraint stress influences spatial recognition memory on a Y-maze differently between the sexes. Based upon prior studies (Diamond et al., 1996; Shors and Dryver, 1992), acute restraint was predicted to impair spatial recognition memory in males and this effect may be observed for females. However, the study by Wood and Shors (1998) implies that the sexes may have opposing responses to acute stress and thus, acute stress may facilitate spatial recognition memory in females. The latter finding was observed in the present research with stressed females performing better on the Y-maze than stressed males. Therefore, Experiment 2 was performed to determine whether acute stress influenced spatial recognition memory differently between females in proestrus and estrus, which represents the estrous phases with the highest and lowest estrogen levels, respectively. We predicted that acute stress would facilitate spatial recognition memory in females in estrus compared to females in proestrus.

2. Methods

2.1. Subjects

Approximately 2-month-old Sprague–Dawley rats were purchased from Harlan (Madison, WI) and were same sex, pair housed in two temperature-controlled chambers. Males and females that were arbitrarily assigned to the stress group were housed in a separate chamber than the nonstressed controls. Rats were maintained on a 12 h light–dark cycle (lights off at 7:00 a.m.), and had ad libitum access to food (Teklad diet 8604, Harlan) and tap water. Arizona State University's Institutional Animal Care and Use Committee approved all procedures and these are in accordance with the applicable portions of the Animal Welfare Act and, *Guide for the Care and Use of Laboratory Animals* by DHHS.

2.2. Experiment 1: Influence of acute stress and sex on Y-maze performance

Forty rats were used: 20 males and 20 females. All females were gonadally intact and were not separated into groups based on stage of estrous cycle. Rats were weighed

weekly until their body weights were 335–355 g for males and 232–235 g for females. This weight range is similar to the weights of control males and females in other studies (Diamond et al., 1999; Viau and Meaney, 1991). A week before Y-maze testing, rats were handled daily.

2.3. Experiment 2: Influence of acute stress and estrous cycle on Y-maze performance

Daily vaginal smears were taken between 9:00 and 11:00 a.m. from 41 gonadally intact, virgin female rats. Smears were stained and categorized by 12:00 p.m. so that restraint began by 12:00 p.m. for rats in the stress condition. Vaginal smears were obtained by dipping a sterile swab (0.6 mm diameter, 0.025 in., Fisher Scientific) in sterile saline and then gently swabbing the vaginal lumen. The swabs were smeared onto labeled glass slides that were previously cleaned with 95% ethanol. The cells were fixed with 95% ethanol and then air dried for 15 min before staining with a hematoxylin and eosin stain. The vaginal smears were inspected and categorized using an Olympus microscope ($\times 40$) according to Long and Evans (1922). Rats in proestrus with the highest estrogen levels showed nucleated epithelial cells. In contrast, rats in estrus showed cornified cells that lacked nuclei. Rats were tested when their estrous cycle was clearly identified as being in proestrus or estrus, and only those rats displaying at least three consecutive 4- or 5-day cycles were used. Vaginal smears were accumulated over 2.5 months with the final number of rats tested in each condition as follows: control–proestrus (CP, $n=9$), control–estrus (CE, $n=9$), stress–proestrus (SP, $n=7$), and stress–estrus (SE, $n=8$).

2.4. Restraint

On the day of Y-maze assessment, rats were transported to a novel room and restrained in wire mesh restraints (7 in. circumference $\times$ 9.5 in. long or 9 in. circumference $\times$ 11 in. long) for 1 h. The two different sizes were used so that rats of various sizes would be confined to a similar degree, which is important because females were smaller than males. During restraint, rats were placed back into their home cage and kept in the novel room. Rats were released after 1 h of restraint and kept in the novel room for one more hour to parallel the 2 h interval used between steroid injection and Y-maze training in our prior studies (Conrad et al., 1999a, 1997). The first trial of the Y-maze occurred 2 h following the start of restraint. In Experiment 1, 1 h restraint occurred within the window of 8:00 a.m. and 1:00 p.m. In Experiment 2, restraint started at 12:00 p.m. and ended by 1:00 p.m. because vaginal smears were required to verify the stage of the estrous cycle and were not completed until 12:00 p.m. Again, the first trial of the Y-maze occurred 2 h following the start of restraint. The investigator restraining the rats was not the same person who handled and tested the rats in the Y-maze.

2.5. Y-maze

Spatial memory was assessed using a Y-maze, which was first described by Dellu et al. (1992) and then subsequently validated as a task requiring hippocampal function (Conrad et al., 1996) and spatial memory (Conrad et al., 1996).

The Y-maze was constructed of black Plexiglas with three identical arms ($50 \times 16 \times 32$ cm) and rat bedding covering the bottom. Overt cues were absent inside of the Y-maze, but large visual objects, such as posters and three-dimensional shapes, were placed around the walls in the room containing the Y-maze.

Y-maze training began by placing a rat in one arm of the Y-maze and allowing it to explore two of the three arms with one arm blocked with black Plexiglas. Rats were tested in pairs with each rat being placed alone in one of two Y-mazes. After 15 min of exploration, rats were transported back to the animal colony in their home cage. The soiled bedding in the Y-maze was mixed between trials to reduce odors as cues. Four hours later, the rats were placed back into the start arm and allowed to investigate all three arms for 5 min. The Y-maze taps into rats' innate tendency to explore novel areas (Ennaceur and Delacour, 1988), and rats with intact spatial memory will enter the novel arm more than the other arm, whereas rats with impaired spatial memory will enter the novel and other arms similarly (Conrad et al., 1996). The arms (start, other, and novel) were randomly assigned and counterbalanced among rats. The investigator was not visible to the rat during Y-maze exploration.

The performance on the Y-maze was videotaped for later quantification. The number of entries made into each arm measured spatial memory and locomotion. An entry was counted when a rat placed half of its upper body into an arm. The testing order of experimental groups was randomized and revealed after quantification.

2.6. Corticosterone measures and assay

In Experiment 1, trunk blood was taken from another set of male and female rats to measure corticosterone levels in response to restraint and Y-maze exposure ($n=4$–6 rats/group). For the baseline measure (Con), rats were rapidly decapitated and trunk blood was taken within 3 min of disturbance. Trunk blood was obtained in the remaining groups by rapid decapitation: immediately following 1 h of restraint (Str), after the completion of the 15 min training trial on the Y-maze (Con-Ymaze), and after the completion of the 15 min training trial on the Y-maze following 1 h restraint (Str-Ymaze).

In Experiment 2, blood was sampled to measure corticosterone levels during and after restraint in females at proestrus and estrus. The same rats tested on the Y-maze were given several weeks after testing before corticosterone levels were determined. Vaginal smears were taken daily between the completion of the Y-maze experiment and the

day that corticosterone was assessed. Once a female rat was determined to be consistently cycling every 4 or 5 days, the rat was restrained during proestrus or estrus. Blood was sampled quickly for the baseline measure by removing the rat from its cage, transporting it to a novel room, and sampling the blood from the tail vein within 3 min of the initial disturbance. Blood from the tail vein was sampled again at 30, 60, and 180 min from the start of restraint. To parallel the restraint duration, rats were removed from restraint after an hour, but kept in the novel room until the last blood sample was obtained.

The timing for blood sampling differed between Experiments 1 and 2 to address separate issues. The purpose of Experiment 1 was to compare corticosterone levels in males and females following restraint, Y-maze and combined restraint and Y-maze. Experiment 2 differed in timing because we wanted to compare the phases of the estrous cycle during restraint that would be consistent with past work (Viau and Meaney, 1991). Moreover, intact females in Experiment 1 showed the most variability in corticosterone levels following restraint, which implies that females in distinct stages of estrus may release unique magnitudes of corticosterone during restraint and/or recovery.

Blood was centrifuged for 30 min at 4000 rpm using a Heraues centrifuge (model = Megafuge 1.0R, VWR), and the serum was removed and stored at −70 °C. Total corticosterone was determined by an enzyme immunoassay (EIA) kit (026-AC-14F1) purchased from American Laboratory Products (Windham, NH). Antibody cross-reactivity to other steroids did not exceed more than 0.05%. Optical density values were measured at 450 nm using a microplate reader (model = LabSystems Multiskan RC, Fisher Scientific). Samples were diluted 1:10 and then processed in duplicate and final values were averaged and represented as microgram per 100 milliliters.

2.7. Data analysis

2.7.1. Spatial memory: dependent variable

The entries made into each arm of the Y-maze during the 5 min were converted into percentages of total entries made into all three arms. The percentage of entries made into the novel and other arms were used in the following analyses. The start arm was not included in these analyses because rats were placed in the start arm and rarely reenter it for their first entry.

2.7.2. Spatial memory: between-group comparison

Analysis of variance (ANOVA) was performed to compare across groups for spatial memory on the Y-maze. The two between-subjects factors were as follows: Experiment 1: treatment (control and acute stress) and sex (male and female); Experiment 2: treatment (control and acute stress) and estrous cycle phase (proestrus and estrus). The dependent variable for spatial memory was a difference score, which was calculated by subtracting the percentage of entries into the other arm from the percentage of entries into the novel arm. Positive values reflect preference for the novel arm and values at zero indicate exploration at chance levels.

2.7.3. Spatial memory: within-group comparison

To further examine whether rats recognize the novel arm for a within-subject comparison, a nonparametric analysis was performed using Wilcoxon matched pairs tests. The percentage of entries into the novel arm was compared to the percentage of entries into the other arm. Rats displaying intact spatial memory will enter the novel arm more than the other arm, whereas rats with impaired spatial memory will enter the novel and other arms similarly.

2.7.4. Motivation

Motivation to explore the maze was compared using locomotion, which was calculated by adding the entries made into all three arms during the 5 min. Locomotion in the Y-maze was compared using a 2×2 ANOVA for the following: Experiment 1, treatment and sex; Experiment 2, treatment and estrous cycle phase. The dependent variable for locomotion was total entries.

2.7.5. Corticosterone levels

For Experiment 1, a 2×2 ANOVA for treatment and sex was performed for serum corticosterone levels. For Experiment 2, a repeated-measures ANOVA was performed for estrous cycle phase (proestrus and estrous) and the repeated measure of time (0, 30, 60, and 180 min).

2.7.6. For all data

Least significant difference (LSD) post hoc tests were used when ANOVA reached significance. Data are represented by means ± S.E.M.

3. Results

3.1. Experiment 1: Influence of acute stress and sex on Y-maze performance

Control males and stressed females exhibited better spatial memory performance on the Y-maze compared to stressed males and control females, as indicated by a significant interaction between treatment and sex [$F(1, 31) = 4.66$, $P < .05$] and subsequent LSD tests (Fig. 1A). The results show that the difference scores were significantly more positive for control males and stressed females than stressed males and control females. The main effects for treatment ($P = .68$) and sex ($P = .94$) were not significant. Wilcoxon nonparametric tests supported these observations with control males entering the novel arm more than the other arm ($P < .05$) and stressed females showing a tendency to enter the novel arm more than the other arm ($P = .07$). In contrast, stressed males and control females

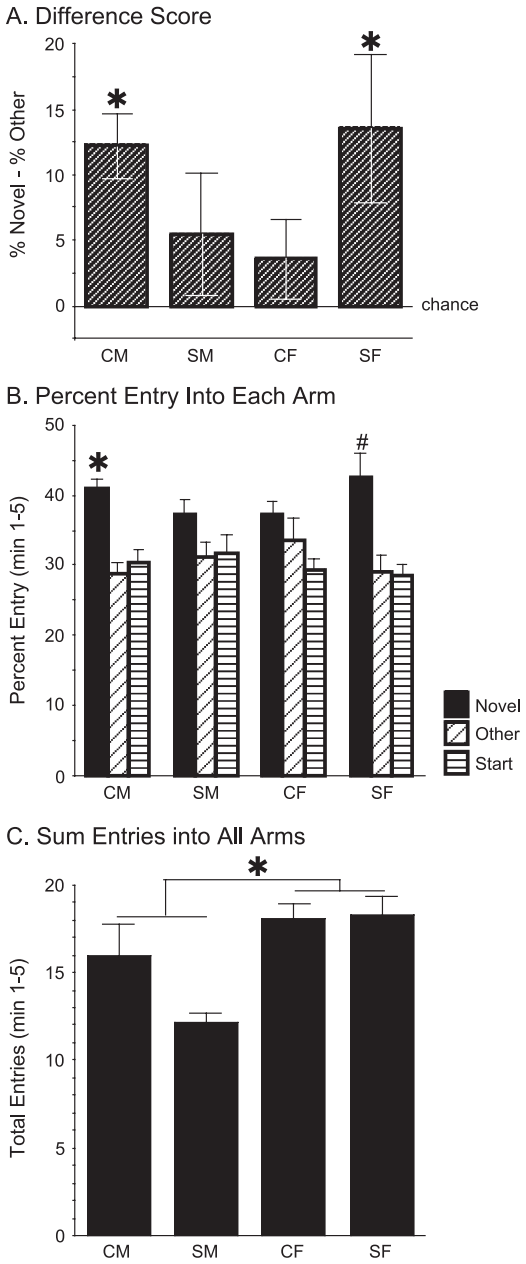

Fig. 1. Influence of acute stress and sex on Y-maze performance. (A) Acute stress for 1 h, followed by training on the Y-maze 2 h after the start of restraint impaired Y-maze performance in males, but not in females [a significant Treatment × Sex interaction, $F(1,31) = 4.66$, $P < .05$, followed by LSD tests]. The greater difference score indicates that more entries were made into the novel arm than the other arm. *$P \leq .05$ compared to SM and CF. (B) Control males entered the novel arm more than the other arm, and stressed females showed a tendency to enter the novel arm more than the other arm. In contrast, stressed males and control females entered the novel and other arms similarly, novel vs. other arm, *$P \leq .05$, #$P = .07$. (C) Control and stressed females entered more arms overall (sum of entries into all three arms) compared to control and stressed males [main effect of sex, $F(1,31) = 11.22$, $P < .01$]. *$P \leq .05$, CF and SF vs. CM and SM. CM = control male, SM = stress male, CF = control female, and SF = stress female.

entered the novel and other arms similarly ($P > .1$, Fig. 1B). Due to an error during testing, five rats were excluded in the analyses, giving rise to the subject numbers: 10 control males, 10 control females, 9 stressed males, and 6 stressed females.

Locomotion was greater for the females compared to males, which was measured by the total entries made into all arms during the 5 min of testing. A two-way ANOVA showed a significant main effect for sex [$F(1,31) = 11.22$, $P < .01$]. LSD tests revealed that both control and stressed females entered more arms than males (Fig. 1C). The main effect for treatment ($P = .16$) and interaction between treatment and sex ($P = .11$) were not significant.

To determine whether locomotion and Y-maze performance correlated during testing, total entries were compared to difference scores between the novel and other arm. None of the correlations were significant in the following conditions: all rats ($r = .017$, $P = .92$), control males ($r = .27$, $P = .48$), control females ($r = -.10$, $P = .85$), stressed males ($r = -.28$, $P = .44$), and stressed females ($r = -.36$, $P = .30$).

A separate group of male and female rats was used to determine the influence of restraint and Y-maze training on corticosterone levels. A 2 × 4 ANOVA was performed using between-subjects measure of sex (male and female) and treatment (baseline, 1 h restraint, control + Y-maze, and 1 h restraint + Y-maze). A significant main effect for treatment was found [$F(3,33) = 17.82$, $P < .001$], with no significant effects for sex ($P = .24$) or interaction ($P = .86$). LSD post hoc tests on treatment showed that all three conditions (1 h restraint, control + Y-maze, and 1 h restraint + Y-maze) had elevated corticosterone levels compared to baseline (Fig. 2). Moreover, restraint significantly elevated corticosterone levels compared to Y-maze testing in control and stressed rats.

3.2. Experiment 2: Influence of acute stress and estrous cycle on Y-maze performance

Regardless of the stage of the estrous cycle, 1 h of restraint enhanced Y-maze performance (Fig. 3A), as shown by a significant main effect for treatment [$F(1,29) = 5.89$, $P < .05$], followed by LSD post hoc tests. Stressed rats exhibited greater positive difference scores than control rats, regardless of estrous phase. Rats in proestrus performed better than rats in estrus, but this effect did not reach statistical significance [main effect of cycle, $F(1,29) = 3.23$, $P = .08$]. The interaction between stress history and cycle was not significant ($P = .57$). Wilcoxon nonparametric tests supported this interpretation as the stressed rats in proestrus and estrus entered the novel arm more than the other arm ($P < .05$). Control rats in proestrus and estrus entered the novel and other arms similarly ($P > .1$, Fig. 3B).

Total entries into each arm were measured to determine whether estrous cycle and stress influenced locomotion/motivation. A main effect for cycle approached significance [$F(1,29) = 3.44$, $P = .07$], with rats in estrus showing a tendency to enter more arms than rats in proestrus (Fig. 3C). The main effect for stress history was not significant

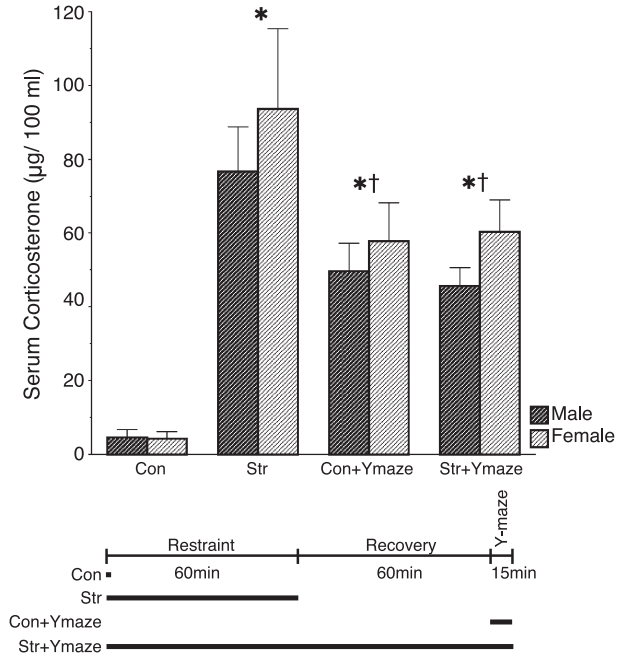

Fig. 2. Influence of acute stress and sex on serum corticosterone levels. One hour of restraint (Str) for males and females significantly elevated serum corticosterone levels compared to controls (Con), controls tested on the Y-maze (Con + Ymaze) and stressed rats tested on the Y-maze (Str + Ymaze). Moreover, Y-maze exposure significantly elevated serum corticosterone levels in control and stressed rats tested on the Y-maze (Con + Ymaze and Str + Ymaze) compared to controls [main effect of treatment, $F(3,33) = 17.82$, $P < .001$]. Although females showed a tendency to exhibit higher serum corticosterone after restraint or Y-maze exposure than males, the main effect of stress and the interaction between treatment and stress did not approach significance (P=.24 and P=.86, respectively). The scale on the bottom represents the treatment condition before blood samples were immediately collected. *$P \leq .05$ compared to Con, $^{\dagger}P \leq .05$ compared to Str. Con = unstressed control rats not exposed to the Y-maze, Str = stressed rats not exposed to the Y-maze, Con + Ymaze = unstressed controls trained on the Ymaze, and Str + Ymaze = stressed rats trained on the Y-maze.

(P=.48), and neither was the interaction between history and cycle (P=.10).

Correlations were performed on total entries and difference scores between the novel and other arm to determine whether a relationship existed between locomotion and spatial memory. Only one correlation was significant for control rats in estrus (r=.83, $P < .01$). As control females in estrus entered more arms, discrimination between the novel and other arm improved. The correlations and P values for the remaining groups were as follows: control + proestrus ($r = -.49$, P=.17), stress + proestrus ($r = -.11$, P=.82), and stress + estrus (r=.045, P=.92).

Several weeks after Y-maze testing, tail blood was taken to assess the influence of estrous cycle and acute stress on corticosterone levels. A 2 × 4 mixed-factor ANOVA with estrous cycle phase (estrus and proestrus) as the between-subjects factor and minute after restraint (0, 30, 60, and 180) as the repeated measure showed a significant effect by minute [$F(3,72) = 81.1$, $P < .001$] and a significant

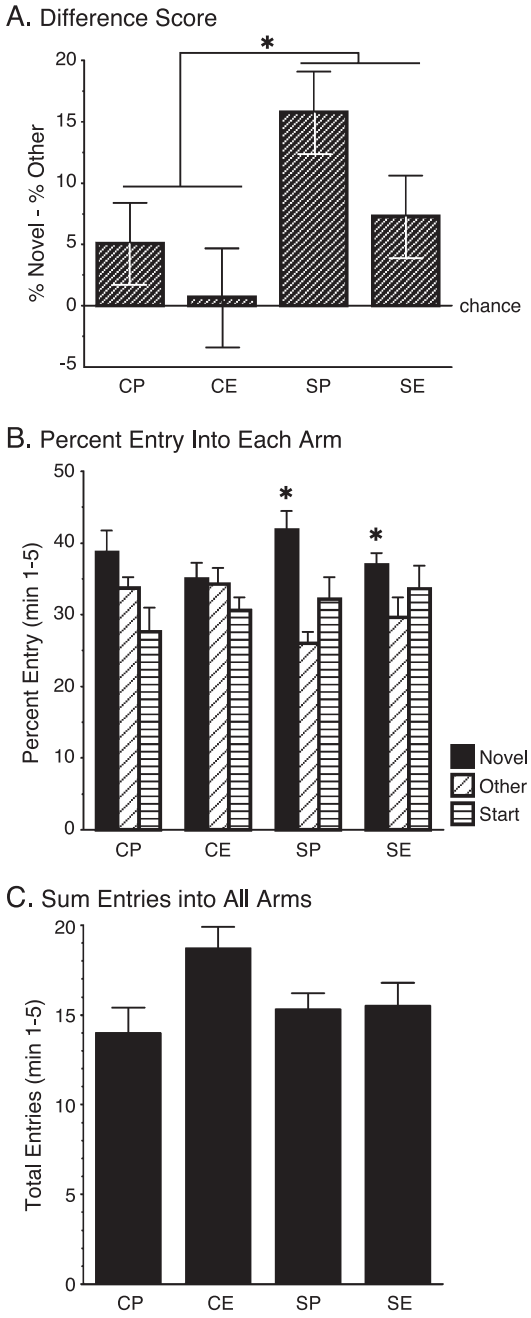

Fig. 3. Influence of acute stress and estrous cycle on Y-maze performance. (A) One hour of restraint enhanced Y-maze performance in rats during both proestrus and estrus [significant main effect for treatment, $F(1,29) = 5.89$, $P < .05$]. A nonsignificant tendency was observed for rats in proestrus to perform better than rats in estrus [main effect of cycle, $F(1,29) = 3.23$, P=.08]. The greater difference score indicates that more entries were made into the novel arm than the other arm. *$P \leq .05$ for SP and SE vs. CP and CE. (B) Wilcoxon nonparametric tests compared the number of entries into the novel arm with the other arm and revealed that stressed rats in proestrus and estrus entered the novel arm more than the other arm. Control rats in proestrus and estrus entered the novel and other arms similarly. *$P \leq .05$ for novel vs. other arm. (C) A tendency was observed for rats in estrus to enter more arms (sum of entries made in all three arms) compared to rats in proestrus [main effect for cycle, $F(1,29) = 3.44$, P=.07]. CP = control proestrus, CE = control estrus, SP = stress proestrus, and SE = stress estrus.

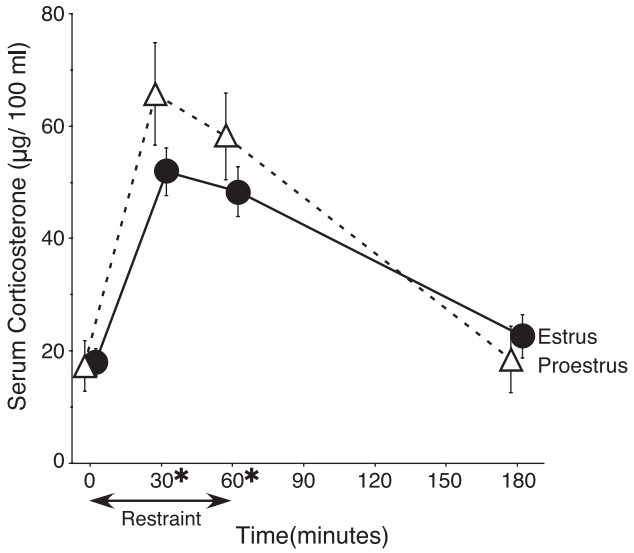

Fig. 4. Influence of acute stress and estrous cycle on serum corticosterone levels. Female rats in proestrus exhibited higher corticosterone levels 30 and 60 min after the start of 1 h restraint compared to rats in estrus [significant Cycle × Minute interaction, $F(3,72)=3.154$, $P<.05$]. Female rats in both conditions of proestrus and estrus exhibited higher corticosterone levels compared to baseline and these corticosterone levels returned to baseline levels 2 h after restraint ended [significant effect by minute, $F(3,72)=81.1$], $*P\leq.05$ for 30 and 60 min compared to 0 and 180 min.

cycle × minute interaction [$F(3,72)=3.154$, $P<.05$]. The effect for cycle was not significant, $P=.47$. LSD tests showed that while rats in estrus and proestrus exhibited similar corticosterone levels at baseline (0 min) and recovery (180 min), rats in proestrus had higher corticosterone levels 30 and 60 min after the start of restraint (Fig. 4).

4. Discussion

The data from Experiment 1 support the hypothesis that acute restraint stress influences spatial recognition memory on the Y-maze differently between the sexes. While 1 h of restraint impaired spatial memory in males, stressed females demonstrated the opposite pattern by exhibiting intact spatial memory. The influence of the estrous cycle and acute stress on spatial memory ability in females was examined in Experiment 2. Acute stress facilitated spatial memory in females during both proestrus and estrus compared to nonstressed controls in proestrus and estrus. These latter data support the overall effect found in Experiment 1 with females showing intact spatial memory after acute stress even without categorizing the females based upon their estrous cycle phase. While Experiment 2 revealed a nonsignificant tendency for females in proestrus to show better spatial memory compared to females in estrus ($P=.08$), the main finding was that acute stress facilitated spatial memory, regardless of the phase of the estrous cycle. The effect of acute stress appeared to be confined to the Y-maze training period, rather than testing because corticosterone levels of females returned to prestress levels within 3 h. Therefore, acute restraint mostly likely influenced acquisition and consolidation of spatial memory processes. Overall, these data demonstrate significant differences in how the sexes perform on the Y-maze after 1 h of restraint.

A potential concern in this study is that acute stress may have influenced performance factors rather than spatial memory because restraint was given prior to training. For instance, acute stress by restraint or predator exposure can be anxiogenic, as measured by decreased open arm entries in the elevated plus maze (Heinrichs et al., 1994; Padovan and Guimarães, 2000; Adamec and Shallow, 1993). These studies may suggest that when restraint precedes training, rats may explore less in paradigms, such as the Y-maze. However, locomotion levels did not predict spatial memory ability in the current study. In Experiment 1, stressed females performed well while control females did not, and yet both groups exhibited elevated locomotion relative to males. Moreover, total entries were similar between control and stressed males, and yet only the stressed males showed impaired spatial memory. In Experiment 2, spatial memory differences were found despite a lack of locomotion differences among groups. The correlations between spatial memory ability and locomotion levels were not significant except for females in estrus that were not exposed to acute restraint. Thus, acute restraint did not appear to alter locomotion, which suggests that acute stress-induced changes in motivation did not contribute to performance differences under these current conditions.

The influence of acute stress on memory performance reveals sex differences, with acute restraint impairing spatial memory in males and facilitating performance in females. In a previous report, we found a similar phenomenon with females, and not males, showing intact spatial memory following chronic stress as indicated by the ability to recognize the novel arm of the Y-maze (Conrad et al., 2003). Others have also demonstrated that chronic stress facilitates a spatial object placement task in females and not males (Beck and Luine, 2002). Moreover, females in proestrus that were stressed for 4 weeks exhibit enhanced radial arm maze performance compared to controls (Bowman et al., 2001). Our findings that acute restraint impairs spatial recognition in males is consistent with another study that found restraint and tailshock impaired object recognition following a 3 h delay, but not a 5 min delay (Baker and Kim, 2002). Other studies have reported the opposite pattern with acute stress facilitating male and not female performance (Johnston and Rose, 1998; Wood and Shors, 1998; Wood et al., 2001). However, these tasks are intrinsically aversive and include training chicks to avoid a noxious tasting bead (Johnston and Rose, 1998), or conditioning rats to blink to a periorbital eyeshock (Wood et al., 2001; Wood and Shors, 1998). As reviewed in several papers (Conrad, in press; Sandi, 1998), highly aversive tasks have different

properties compared to less aversive tasks (such as the Y-maze), and the neural substrates underlying these behaviors are not identical. Thus, acute stress may influence the sexes differently by facilitating male performance on highly aversive tasks, and female performance on less aversive spatial tasks.

Our current study also supports the interpretation that control males exhibit better spatial memory than control females. Previous studies have reported that males tend to perform better than females on spatial mazes (Beatty, 1992). In an earlier study, however, control females performed as well as control males in the Y-maze (Conrad et al., 2003). Although estrous cycles were not determined, one interpretation is that the earlier study contained fewer females in estrus compared to other estrous stages, skewing the results toward more intact spatial memory for the females. Another report compared water maze performance among males, females in proestrus (behavioral estrus), and females in diestrus (Frye, 1995). Females in proestrus performed worse than males and females in diestrus, while females in diestrus performed as well as males. Additionally, the timing between training and testing may be a critical factor in detecting sex differences on memory tasks. A report in this issue of *Pharmacology, Biochemistry and Behavior* shows that sex differences were not present when inhibitory avoidance assessment took place 24 h later when the stage of the estrus cycle in females differed between training and testing (Rhodes and Frye, 2004). In contrast, females in proestrus showed better inhibitory avoidance compared to males and diestrous females following a 4-h delay, when training and testing occurred within the same phase of the female estrous cycle (Rhodes and Frye, 2004). Overall, these reports indicate that sex differences on spatial memory ability can be subtle and may vary depending on several factors, including the stage of learning and/or memory processing and stage of estrous cycle.

Serum corticosterone levels were measured to determine whether sex and estrous cycle influenced the stress response to restraint and the Y-maze. Both sexes responded to restraint, Y-maze, and the combination of restraint and Y-maze similarly by showing a robust corticosterone response after restraint and a moderate corticosterone response after Y-maze exposure. Interestingly, restraint followed by Y-maze exposure produced corticosterone levels that were similar to those produced by Y-maze exposure alone. Perhaps the elevated corticosterone levels released in response to restraint had already begun to negatively feedback and inhibit corticosterone responses that would have been produced by another event, such as Y-maze exposure. Hence, corticosterone levels following the combined exposure to restraint and Y-maze would not be additive. Although females appeared to exhibit higher corticosterone levels to restraint and the Y-maze compared to males, this effect did not approach significance (P=.24). These data indicate that corticosterone levels secreted in response to the Y-maze were not the sole mechanism underlying sex differences in Y-maze performance following acute restraint. Another study found that 1 h following the start of 30 min restraint, females in proestrus and estrus had similar corticosterone levels compared to males (Figueiredo et al., 2002). These findings support our current results because females as a group may not necessarily exhibit higher corticosterone levels compared to males. Other studies have reported stress-induced sex differences in corticosterone levels with females exhibiting higher levels of corticosterone than males after a variety of stressors and delays ranging from 5 min to 24 h (Haleem et al., 1988; Kant et al., 1983; Le Mevel et al., 1979; Livezey et al., 1985; MacLusky et al., 1996; Shors et al., 2001; Wilson and Biscardi, 1994). One report found the opposite with male rats secreting twice as much corticosterone than females 60 min after confinement in a glass cylinder (Karandrea et al., 2000). However, the Wistar rat strain was used, which contrasts with the majority of reports that studied the Sprague–Dawley strain. This difference may arise because species and strain can greatly influence glucocorticoid levels and hippocampal function (Cabib et al., 1996; Paré, 1996). While the data in our current study support the tendency for acute stress to elevate corticosterone levels in Sprague–Dawley females to a greater extent than to males, the literature indicates that these effects may be subtle and dependent upon the stage of the estrous cycle.

In the current study, the estrous cycle was quite potent in altering serum corticosterone levels following restraint, while sex only moderately altered corticosterone levels. Females in proestrus showed a higher magnitude of corticosterone levels during restraint compared to females in estrus, while no differences in corticosterone levels were observed at baseline and after 2 h of recovery. These data are consistent with other reports that found cycling female rats in proestrus had significantly higher corticosterone levels than females in estrus following 20 min restraint (Viau and Meaney, 1991). These findings also imply that estrous cycle should have influenced Y-maze performance. However, a significant interaction between stress treatment and cycle was not observed, indicating that factors associated with proestrus appeared to enhance performance of both stressed *and* nonstressed females and suggests that the stress-induced release of corticosterone was not the sole determinant of performance. For baseline corticosterone levels, however, another report showed that nonstressed females in proestrus had higher corticosterone levels than females in estrus and that females in proestrus and estrus showed similar levels of corticosterone 60 min after restraint (Figueiredo et al., 2002). Primary differences between these studies were that we used wire mesh restraints for 60 min, compared to 30 min in plastic restraint tubes used by Figueiredo et al. Moreover, blood was taken repeatedly via tail vein from the same rats in our study, whereas Figueiredo et al. sampled once using trunk blood. Thus, the restraint and blood sampling procedure we used may

have been more aversive than that used by Figueiredo et al. In summary, total levels of serum corticosterone were not the sole mechanism that allowed stressed females to perform well on the Y-maze after 1 h of restraint.

The mixed results for how the estrous cycle affects memory may reflect the influence of the estrous cycle on search strategies. For example, our data suggested a non-significant tendency for females in proestrus to show better spatial memory on the Y-maze than females in estrus, which contrasts to several reports. Some showed that estrous cycle did not alter spatial working memory on the radial arm maze (Stackman et al., 1997) and swim task (Berry et al., 1997). Others found that females in estrus performed better than those in proestrus on a spatial version of contextual fear conditioning (Markus and Zecevic, 1997) and swim task (Warren and Juraska, 1997). In contrast to hippocampus-sensitive spatial tasks, one report found that females in proestrus performed better than females in estrus on a cued version of the swim task (Warren and Juraska, 1997). Taken together, these findings suggest that estrogen may bias the strategy used to navigate, depending upon whether the task is spatial or nonspatial. This hypothesis was recently tested in ovariectomized females replaced with estrogen (Korol and Kolo, 2002). The results indicated that estrogen improved learning in ovariectomized rats compared to ovariectomized untreated controls tested in a place (spatial) task, whereas ovariectomized controls learned more quickly on the response (nonspatial) task compared to estrogen-treated rats. Our current data match the findings of Korol and Kolo (2002), with rats in proestrus (high estrogen) showing better spatial recognition memory on the Y-maze than rats in estrus (low estrogen). The studies as a whole may suggest that females shift navigation strategies during the course of the estrous cycle, and that estrous cycle may influence learning in a task-dependent manner.

In summary, these data show that males and females respond to acute restraint stress differently with spatial recognition memory being impaired in males and facilitated in females. In parallel with these results, young adult male, but not female, volunteers show elevated glucocorticoids with the anticipation of speaking in public (Kirschbaum et al., 1992). Moreover, the levels of glucocorticoids secreted in response to public speaking negatively correlate with declarative memory in males, but not females, representing cognitive functions shown to involve the hippocampus (Wolf et al., 2001). Both the human literature and the current report show that psychological stress in males impairs cognitive function under low aversive conditions (declarative memory in humans and Y-maze spatial recognition memory in rats). In contrast, females are resistant to this type of memory impairment following psychological stress. Moreover, the assessment of memory during proestrus or estrus did not influence the outcome of acute stress on spatial memory performance under these conditions. These data indicate important differences in how the sexes respond to both stress and cognitive tasks.

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