Available online at www.sciencedirect.com

SCIENCE DIRECT®

Pharmacology, Biochemistry and Behavior 78 (2004) 541–550

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Chronic stress alters amphetamine effects on behavior and synaptophysin levels in female rats

Veronica Bisagno[a,1], Claudia A. Grillo[b], Gerardo G. Piroli[b], Paola Giraldo[a], Bruce McEwen[b], Victoria N. Luine[a,*]

[a]Department of Psychology, Hunter College, 695 Park Avenue, New York, NY 10021, USA
[b]Laboratory of Neuroendocrinology, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

Received 2 February 2004; received in revised form 22 April 2004; accepted 27 April 2004

Abstract

Previous studies show that stress cross-sensitizes with or alters amphetamine (AMPH) effects in male rats; however, few studies include females. We investigated combining daily restraint stress (21 days for 6 h/day) with chronic AMPH (10 injections every other day) on locomotor activity, exploratory activity in an open field and object recognition, a memory task, in female rats. A synaptic protein, synaptophysin, was also quantified by radioimmunocytochemistry (RICC) in brain to determine possible mechanisms for behavioral changes. Beginning at 5 days after cessation of treatments, AMPH increased locomotion, modified exploration, impaired object recognition, and increased serum corticosterone (CORT) levels. Stress did not alter these parameters but blocked AMPH effects on exploration and object recognition, potentiated AMPH-dependent locomotor effects, and did not alter increased CORT levels. AMPH treatment decreased synatophysin expression in the hippocampus. In the caudate nucleus, the AMPH group showed increased synaptophysin expression which was reversed by stress. These results in females corroborate previously shown cross-sensitizations between stress and AMPH for locomotion in males and demonstrate that chronic stress counteracts AMPH-dependent impairments in recognition memory. Stress may counteract AMPH effects on the memory task by blocking both the induction of AMPH anxiety-like effects and neuroplastic changes in the caudate nucleus of female rats.

Keywords: D-amphetamine; Stress; Female; Rats; Object recognition; Locomotion; Synaptophysin; Caudate; Hippocampus

1. Introduction

In experimental animals, sexual dimorphisms have been reported in the initial response to psychomotor drugs and following repeated drug exposure. Locomotor activity and stereotypical components of behavior are higher in female rats in comparison with male rats following either acute or chronic amphetamine (AMPH) treatments (Camp and Robinson, 1988a,b; Bisagno et al., 2003a,b; for review, see Becker, 1999). In addition, females show increased motivation for self-administration of cocaine and methamphetamine (Lynch and Carroll, 1999; Roth et al., 2002) and enhanced sensitivity to conditioned place preference for cocaine than males (Russo et al., 2003). In humans, women, as compared to men, show higher levels of subjective ratings of "euphoria" after acute AMPH and a more rapid onset of cocaine addiction (Lynch and Carroll, 1999).

Sex differences are also present both in behavioral and neurochemical responses to acute and chronic stress. Following acute stress, females show enhanced performance on the Y-maze memory task while performance of males is impaired (Conrad et al., 2004). Following chronic stress, females show either no changes in or enhanced performance of memory tasks while males are impaired by the same stress regimen (Luine, 2002; Bowman et al., 2001; Conrad et al., 2003). Males and females also respond differently to stress in conditioning

* Corresponding author. Tel.: +1-212-772-4223; fax: +1-212-772-5620.
E-mail address: vluine@hunter.cuny.edu (V.N. Luine).
[1] Present address: Instituto Cajal, CSIC, Av. Dr. Arce 37, 28002 Madrid, Spain.

0091-3057/$ – see front matter © 2004 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2004.04.023

paradigms. Stressed male rats acquire associative conditioning faster than controls whereas females exposed to the same stress are learning impaired (Wood and Shors, 1998). The differences in responses to stress between the sexes appear to be due to a combination/interaction of both developmental and activational factors (Luine, 2002).

Although the consequences of chronic drug administration on learning and memory have been less studied, our laboratory has previously found that male rats are more sensitive than females to effects of chronic AMPH treatment on performance of a recognition memory task, object recognition (Bisagno et al., 2003a,b). In males, stress potentiates reactivity to both the rewarding and locomotor properties of psychoactive drugs (Piazza and Le Moal, 1996). Stress and psychostimulants share common brain circuits and effects on neurotransmitters (Alexander et al., 1990; Koob et al., 1998). Recent studies also indicate convergence of these stimuli on the morphology of dendrites and spines. Chronic exposure of males to AMPH or cocaine produces increased dendritic branching, density of synaptic spines and the number of branched spines in pyramidal neurons of the frontal cortex and nucleus accumbens (Robinson and Kolb, 1997) while stress induces decreased dendritic branching in hippocampal and frontal cortex pyramidal neurons of males (Woolley et al., 1990; Silva-Gomez et al., 2003).

Most effects of psychoactive drugs or stress have been characterized following treatments to male rats, and it is thus unclear whether female rats show the same changes as male rats. The aim of the present study was to determine possible interactions between chronic stress and AMPH treatment in female rats. We measured locomotion, exploratory activity, and object recognition in female subjects. Because both psychostimulants and stress induce profound changes in spine density and dendritic remodeling, the expression of the synaptic protein, synaptophysin, was measured in several CNS areas involved in memory processes and the reinforcing effects of drugs: hippocampus, caudate nucleus, and the nucleus accumbens.

2. Materials and methods

2.1. Animals

Intact adult female Sprague–Dawley rats (190–200 g) were obtained from Harlan, housed in pairs under a 14:10 light/dark cycle (lights on 7:00 a.m.) with water and food ad libitum. After an acclimation period of 2 weeks, rats were randomly assigned to the experimental groups.

All animal use followed the NIH *Guide for the Care and Use of Laboratory Animals* and the experimental protocol was approved by the Institutional Animal Care and Use Committee at Hunter College of the City University of New York.

2.2. Drug treatment/experimental procedure

For chronic AMPH treatment, the experimental animals received an intraperitoneal injection of 2.6 mg/kg D-amphetamine sulfate (AMPH; Sigma, St. Louis, MO, USA), dissolved in 0.9 % sterile saline (volume: 1 ml/kg body weight) once every 2 days for a total of 10 injections. Controls received the same volume of 0.9% sterile saline. The experimental groups were as follows: control (10 injections of saline, $n=10$), AMPH (10 injections of 2.6 mg/kg of AMPH, $n=10$), stress (21 days of 6 h/day restraint stress in a plastic tube and 10 injections of saline, $n=8$), and stress + AMPH (21 days of 6 h/day restraint stress and 10 injections of 2.6 mg/kg of AMPH, $n=8$). Injections were given following the restraint session. It has been shown that this treatment to female rats causes sensitization to a challenge dose of AMPH (Camp and Robinson, 1988a).

2.3. Restraint stress

Subjects were taken daily to an adjacent room where they were placed in Plexiglas restrainers and housed in a sound-attenuating chamber (78-dB white noise was supplied by inflow and outflow fans). Subjects remained in the chamber for 6 h of restraint (9:30 a.m.–3:30 p.m.), a paradigm previously applied in a variety of studies (Magariños and McEwen, 1995; Galea et al., 1997; Bowman et al., 2001; Conrad et al., 2003; Luine, 2002).

2.4. AMPH-induced locomotion

Locomotor activity was quantified over 5-min intervals for 2 h on the 21st day of treatment (immediately following the last AMPH injection). Photocell chambers were affixed to the home cage, 10 in. (w) × 16 in. (l) × 16 in. (h), and simultaneously operated by computer (San Diego Instruments, San Diego, CA). Animals were habituated to the test apparatus for 30 min prior to drug administration. Testing was initiated immediately after the injection.

2.5. Open-field test

Behavioral evaluations began with open-field testing. The field was made of black Plexiglas and had 60-cm-high walls. The floor was divided into 15 equal (20.5 cm each) squares (5 × 3). In subsequent trials with objects on the field (object recognition memory testing), the arena was shortened to nine squares (3 × 3). Approach/avoidance to enter the field (entry latency) was tested by placing rats in a small plastic tunnel connected to the field via a guillotine door. Grid crossings (moving from one square to another) in both outer and inner squares, rears, wall climbs, and defecations were recorded for 6 min by a trained observed blind to experimental conditions. The field was lighted by ambient, low-level, overhead, room lights.

2.6. Object recognition test

Rats usually display a preference for investigating novel over familiar objects. Thus, the object recognition task uses spontaneous exploratory activity and can provide a measure of memory function when a delay interval is interspersed between the initial exploration of objects (sample trial) and the introduction of a new object for exploration (recognition trial; Ennaceur and Delacour, 1988). When rats fail to discriminate between the old and new objects, it indicates impaired memory of the old object.

Object recognition testing consists of two 3-min trials: a sample trial (T1) and a recognition trial (T2) which are separated by an intertrial interval. The total time subjects spend interacting with the objects is recorded. In these trials, two identical objects were placed equidistant from the north corners during T1, but for T2, one of the objects was switched for a new object. The intertrial delay between T1 and T2 was 4 h. Prior to testing, subjects received habituation trials with 10 min-, and 1- and 2-h intertrial delays. Objects used were a variety of soda cans and plastic bottles; both the objects and the field were carefully cleaned between trials. Open-field and habituation trials were conducted before the object recognition tests. Trials were performed between 10:00 a.m. and 5:00 p.m. The left/right locations of the novel object (and which object was the sample or the novel) were fully counterbalanced across groups. Exploration was defined as facing the object (within 2 cm of the object), touching the object (while facing it), sniffing the object, or whisking the object. Data were collected by a blinded observer and are expressed as total exploration time in T1 (in seconds, mean ± S.E.M.) and time spent with the old and novel objects in T2 (in seconds, mean ± S.E.M.; see Bisagno et al., 2003a,b for further details).

2.7. Corticosterone measurement

Five days after the last stress session and AMPH injection, and immediately following the object recognition test, animals were taken to a separate room and sacrificed by decapitation. Trunk blood was collected. Circulating corticosterone (CORT; μg/dl) was measured in serum using coat-a-count radioimmunoassay (DPC, Los Angeles, CA, USA).

2.8. Neurochemical analyses

Following collection of trunk blood, subjects' brains were quickly removed and immediately placed in dry ice. The brains were subsequently stored at −70 °C until radioimmunocytochemistry (RICC) assay. The general methods for synaptophysin RICC are as described by Masliah et al., 1990; Rashid et al., 1995 and previously applied in the laboratory by Brake et al., 2001. Coronal sections were cut at 16 μm on a freezing–sliding microtome, thawed, and then fixed with 4% formaldehyde in 0.05 M PBS for 30 min at room temperature (RT). Sections were washed and incubated with 1% BSA in 0.05 M PBS for 30 min at RT to reduce nonspecific binding. Sections were incubated with antibody against synaptophysin (1:8000, Sigma) for 2 h at RT. Sections were washed with 1% BSA/0.05 M PBS and incubated with [^{35}S]-labeled anti-rabbit antisera at a dilution of 1:400 in 1% BSA/0.05 M PBS for 2 h at RT. Sections were rinsed, dehydrated, air dried, and exposed to Kodak X-OMAT films. Computer-assisted microdensitometry of autoradiographic images (see Fig. 1) was determined on the MCID image analysis system (Imaging Research, St. Catherines, Canada). The values obtained represent the average of measurements taken from two to three sections per slide and two slides per animal.

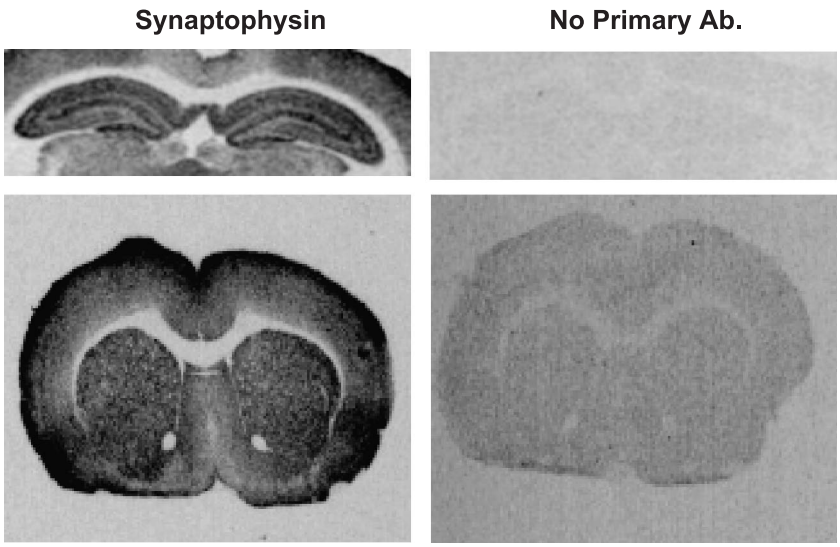

Fig. 1. Autoradiograms of synaptophysin immunoreactivity in female rat hippocampus and caudate/striatum detected using RICC. No primary Ab represents binding of [^{35}S]-labeled anti-rabbit secondary antibody in the absence of primary antibody.

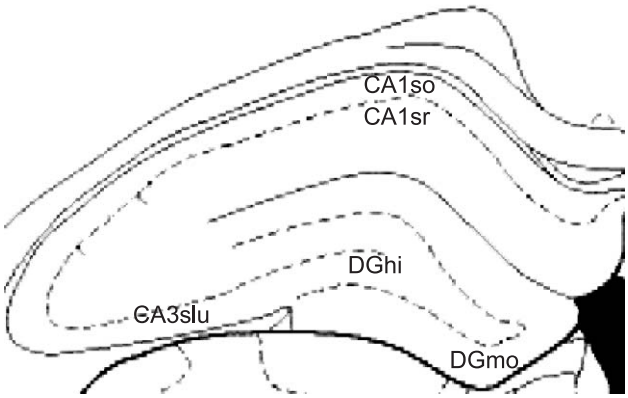

Fig. 2. Schematic diagram identifying specific hippocampal regions where measures were taken. DGhi: dentate gyrus hilus; DGmo: dentate gyrus stratum molecular; CA3slu: CA3 stratum lucidum; CA1so: CA1 stratum oriens; CA1sr: CA1 stratum radiatum.

Synaptophysin expression was quantified in hippocampal areas CA1 (*stratum oriens* and *radiatum*), CA3 (stratum lucidum), and dentate gyrus (hilus and stratum lucidum; see Fig. 2 for schematic diagram). For quantification and analysis, the caudate/striatum was divided into several areas: dorsomedial (DM), dorsomediolateral (DML), dorsolateral (DL), ventromedial (VM), ventromediolateral (VML), ventrolateral (VL), nucleus accumbens core (NacC), and nucleus accumbens shell (NacS) according to Bergstrom et al. (2001). (See Fig. 3 for schematic diagram.)

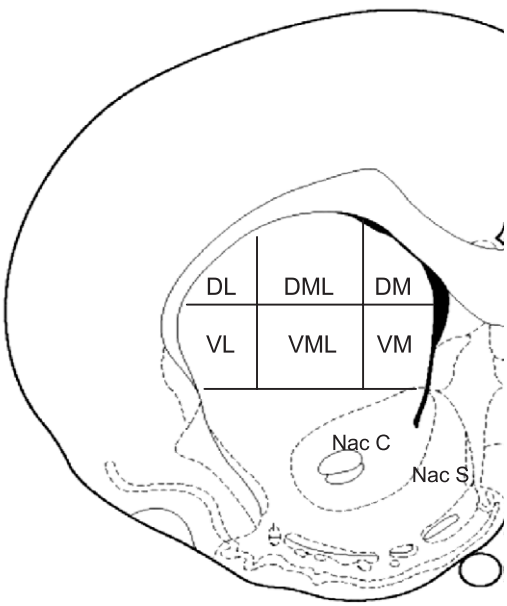

Fig. 3. Schematic diagram identifying specific caudate/striatum regions from where measures were taken, according to Bergstrom et al. (2001). DL: dorsolateral caudate; DML: dorso–medial–lateral caudate; DM: dorso–medial caudate; VL: ventro–lateral caudate; VML: ventro–medial–lateral caudate; VM: ventro–medial caudate; Nac C: nucleus accumbens core; Nac S: nucleus accumbens shell.

2.9. Data analysis

Animal cages were coded for behavioral testing, and analyses were performed by an observer unaware of the rat's treatment condition; synaptophysin films were also coded and analyzed blind. Two-way ANOVA was used to evaluate effects (Stress × AMPH) on locomotion, open field, CORT levels, and synaptophysin expression. Fisher's LSD Test was used as a post hoc test when appropriate. For object recognition, two-way ANOVA (Stress × AMPH) was utilized to test for differences in exploration time during T1. For the recognition trial, T2, two-way ANOVA was used to analyze effects (Group × Object) and paired *t* tests on each group tested whether time spent with the old object was less than time spent with the new object. For all statistics, a significance level of $P < .05$ was established.

3. Results

3.1. Behavioral results

3.1.1. Locomotion

Locomotion effects are reported in Fig. 4. Locomotion was quantified for 2 h in four groups immediately following the last stress session and AMPH injection in the subject's home cage. Two-way ANOVA (Stress × AMPH) revealed an AMPH effect: $F(1,32) = 35.3$, $P < .00001$, a stress effect: $F(1,32) = 5.54$, $P < .03$, and an interaction effect: $F(1,32) = 5.2$, $P < .04$. LSD tests revealed that AMPH-treated rats showed more counts than control ($P < .05$), stressed rats were not different from control, and the stress + AMPH group showed more counts than any other group ($P < .05$),

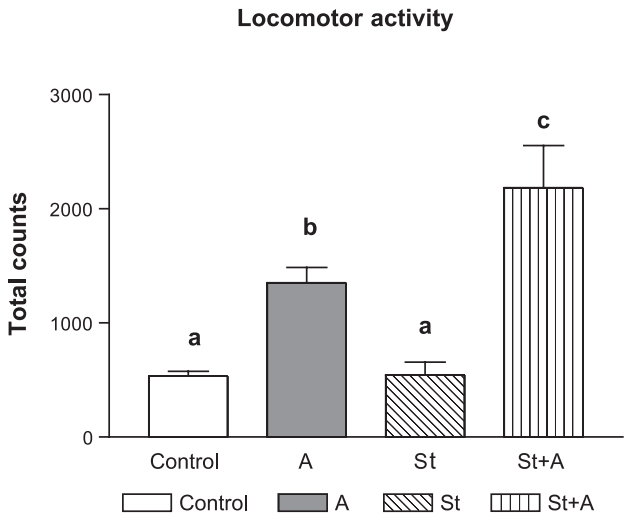

Fig. 4. Effect of chronic AMPH treatment on locomotion in the home cage. Total activity counts accumulated over a 2-h session following the 10th (and last) AMPH injection (2.6 mg/kg) are shown. Bars represent the mean time (± S.E.M.). Groups with different symbols as superscripts are significantly different from each other, $P < .05$ (ANOVA followed by Fisher's LSD).

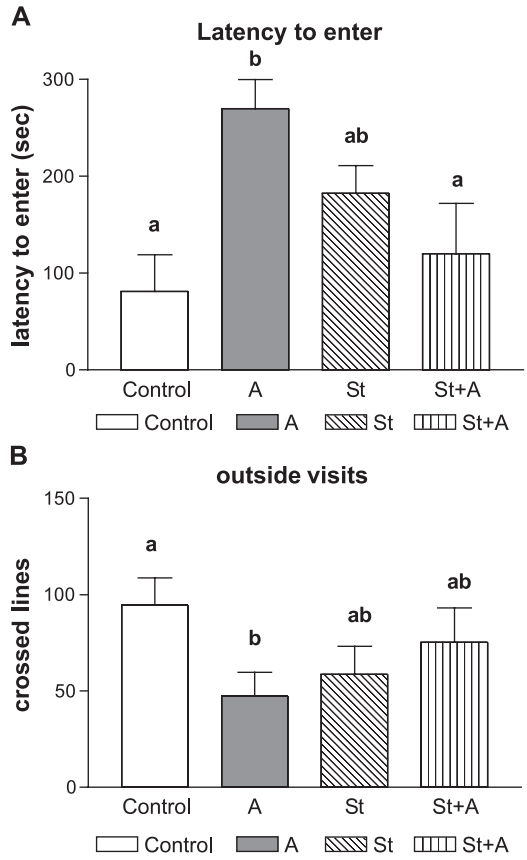

Fig. 5. Exploratory activity in the open field. Open-field activity was recorded 24 h after the last AMPH injection and last stress session. Bars represent means (± S.E.M.). (A) Latency to enter the field from the additional plastic tunnel. AMPH group showed the highest latency to enter the open field. (B) Outside visits. AMPH group showed less visits than the control group. Groups with different symbols as superscripts are significantly different from each other, $P<.05$ (ANOVA followed by Fisher's LSD).

suggesting an additive or synergistic effect of combining stress and AMPH treatment.

3.1.2. Open-field/exploratory activity

In the open-field trial (24 h after the last stress session and AMPH injection), subjects' activities (sector visits, rears, and wall climbs) were recorded for 6 min (Fig. 5). The latency to enter the field, a measure of anxiety, was also measured. Two-way ANOVA indicated a significant interaction effect on time to enter the field [Stress × AMPH, $F(1,32)=10.9$, $P<.01$]. LSD testing indicated that AMPH treatment increased the latency to enter as compared to control ($P<.05$) and the stress group was intermediate between and not different from all groups. The AMPH+stress group was not different from control, suggesting that stress blocked the AMPH increase in latency. For outside visits, two-way ANOVA showed a significant interaction effect [Stress × AMPH, $F(1,32)=4.74$, $P<.05$], and LSD testing indicated that the AMPH group had less visits than the control group, $P<.05$. The stress and stress+AMPH groups were intermediate between and not different from the control or AMPH groups, suggesting that stress antagonized the AMPH effect. The AMPH group was the only group that showed defecations during the open-field test, and no differences among groups in the other parameters measured were noted. Overall, AMPH treatment decreased approach to, and exploration of, the open field, an effect that was blocked or diminished by stress.

3.1.3. Object recognition test

Habituation of the subjects to object recognition trials began 48 h following the last stress session and AMPH injection. No drug injections were given during the 5 days of behavioral testing and habituation trials. Object recognition was tested with a 4-h delay between the sample and

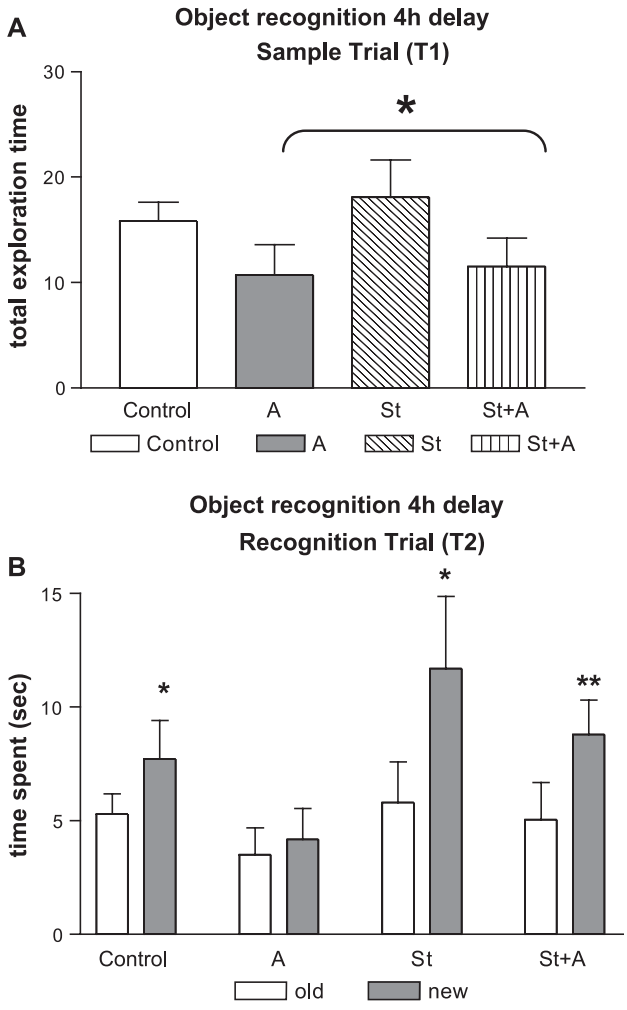

Fig. 6. Object recognition memory after chronic AMPH and stress treatments (5 days after last AMPH/stress session). (A) Total exploration time in the sample trial (T1), AMPH-treated rats (AMPH and stress+AMPH) showed less exploration time in the sample trial than saline groups (control or stressed), $P<.05$ (ANOVA followed by Fisher's LSD Test). (B) Exploration time in the recognition time. Bars represent the mean time (± S.E.M.) exploring the old and the new object in the recognition trial (T2), $**P<.01$, $*P<.05$ (ANOVA followed by paired t test) within each group.

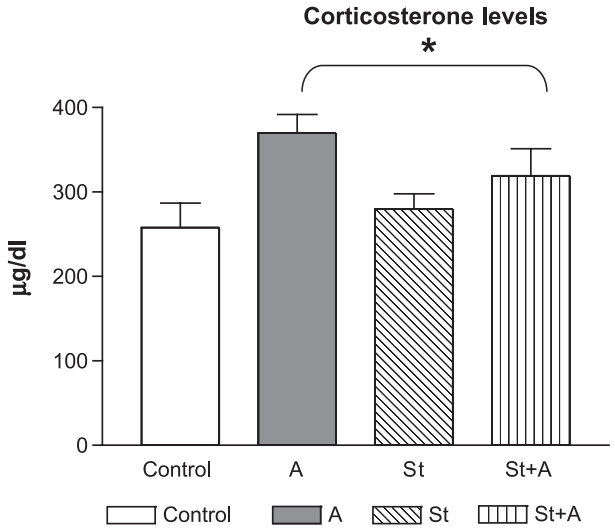

Fig. 7. CORT levels. Samples were taken at sacrifice (5 days after last AMPH/stress session). Bars represent means (± S.E.M.). AMPH-treated groups (AMPH and stress + AMPH) showed higher levels than saline-injected groups (control or stressed), $P < .05$ (ANOVA followed by Fisher's LSD).

recognition trial on the fifth day after cessation of treatments (Fig. 6). Total exploration time in sample trial (T1) was analyzed by two-way ANOVA and showed a significant AMPH effect: AMPH-treated rats showed less exploration time in the sample trial, $F(1,32) = 4.43$, $P < .05$ (Fig. 6 inset).

Comparison of the time spent with the old and the new objects within groups in the recognition trial (T2) by ANOVA showed a significant difference in time spent with the objects, $F(1,229) = 87.41$, $P < .0001$. Paired t tests indicated that the control group ($T = 1.9$, $P < .05$), the stressed group ($T = 2.6$, $P < .05$), and the group that received the combination of stress and AMPH ($T = 8.3$, $P < .01$) spent significantly more time exploring the new object than the old object (Fig. 6). In contrast, the only group that did not show a significant difference between exploration of the old and the new object was the AMPH group; this pattern of results suggests that stress blocked the AMPH-dependent effect on time spent with the objects.

3.1.4. CORT measurement

Analysis of CORT levels indicated (using a two-way ANOVA) an AMPH effect: AMPH-treated rats (AMPH and stress + AMPH) showed higher CORT levels than saline-injected subjects (control or stressed subjects), $F(1,30) = 8.39$, $P < .01$ (see Fig. 7).

3.1.5. Synaptophysin expression

As shown in Fig. 8, AMPH-treated groups (AMPH and stress + AMPH) showed a small, but statistically significant, decrease of synaptophysin expression in the hippocampus,

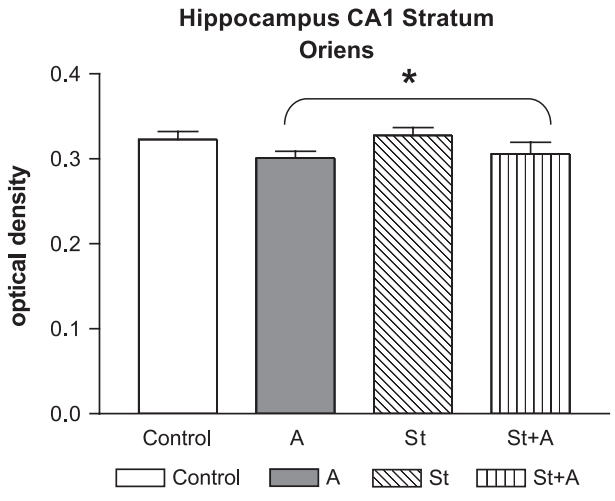

Fig. 8. Synaptophysin expression in hippocampus CA1 area, stratum oriens. Mean (± S.E.M.) of optical density. AMPH-treated groups (AMPH and stress + AMPH) showed lower levels than animals that received saline injections (control or stressed), $P < .05$ (ANOVA followed by Fisher's LSD Test).

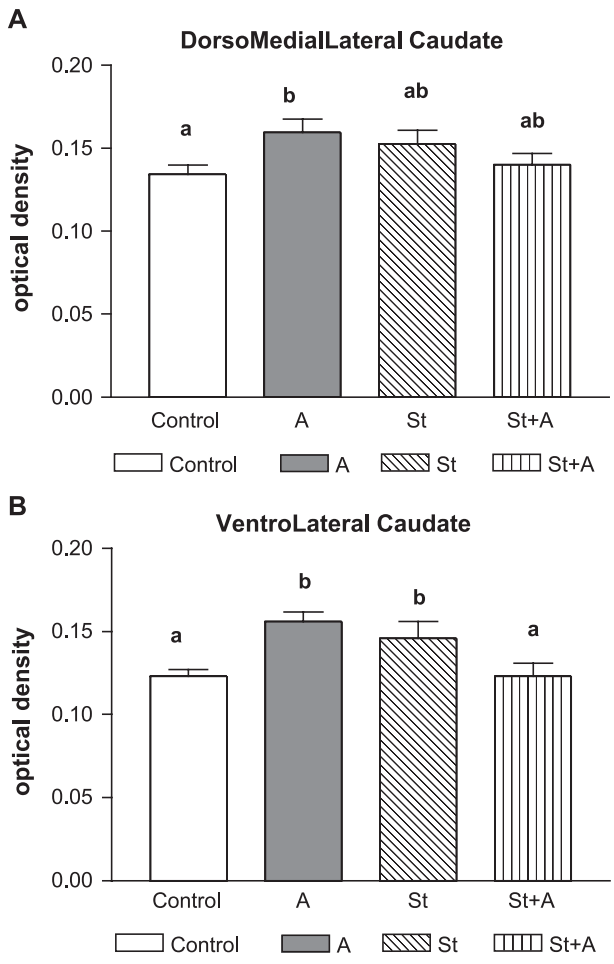

Fig. 9. Synaptophysin expression in the caudate–putamen. Mean (± S.E.M.) of optical density. (A) Dorsomediallateral area, AMPH group showed higher density than the control group, $P < .05$. (B) Ventrolateral area, the AMPH and stressed groups were not different from each other and showed higher levels of synaptophysin than control and stress + AMPH groups, $P < .05$ (ANOVA followed by Fisher's LSD). Groups with different symbols as superscripts are significantly different.

CA1 stratum oriens [two-way ANOVA, $F(1,32)=4.69$, $P<.05$]. No differences were found in other hippocampal areas. In two of six areas examined in the caudate–putamen, AMPH increased synaptophysin expression and stress either antagonized or blocked the increase (see Fig. 9). In the DML region, there was an interaction effect [Stress × AMPH: $F(1,32)=6.74$, $P<.05$]. LSD testing a posteriori indicated that the AMPH group had higher expression than the control group, $P<.05$; the stress and stress + AMPH groups were not different from the control or AMPH groups, suggesting that stress antagonized the AMPH-dependent increase in synaptophysin levels. In the VL region, ANOVA also showed an interaction effect [Stress × AMPH: $F(1,32)=12.76$, $P<.01$], and LSD testing indicated that the AMPH group showed higher levels of synaptophysin expression than control and stress + AMPH groups and that the stress group showed higher levels than the control group ($P<.05$), a pattern suggesting that stress in combination with AMPH blocks the increase in synaptophysin by either treatment alone. No group differences were found in the nucleus accumbens.

4. Discussion

In the present study, we examined the effects of chronic AMPH injections, daily restraint stress, and their combination on a number of neural functions in female rats. Chronic restraint stress potentiated the locomotor-enhancing effects of AMPH. Stress, in combination with AMPH, has been previously shown to enhance the locomotor effects of AMPH; however, this effect was reported only in male rats and mice (Badiani et al., 1992; Covington and Miczek, 2001; Lin et al., 2002; Barr et al., 2002). Here, we report that this effect is also present in female subjects. In contrast, spontaneous exploratory activity in a novel environment, the open field, was inhibited by AMPH, an anxiogenic-like effect. Chronic stress counteracted this AMPH effect on the open field. The AMPH-treated group was also the only group that showed defecations, another indication of anxiety. While open-field measures are not the best parameters for evaluating anxiety responses, the pattern of effects suggests that chronic AMPH induces fear toward a novel, open arena. Clearly, further investigations of possible AMPH and stress effects on anxiety are necessary utilizing anxiety specific tasks, like the plus maze and/or defensive prod burying, to confirm the current effects (Wilson et al., 2004).

In a drug-free state, 5 days after the last injection of a chronic regimen of AMPH, we found that AMPH impaired object recognition. AMPH-treated female rats spent the same amount of time exploring old and novel objects in a recognition trial, suggesting an impairment in memory. Subjects receiving a combination of stress + AMPH performed as well as the control group or the stress group, all of which spent more time exploring the new object than the old object. Thus, similar to anxiety-like parameters on the open field, stress given in combination with AMPH blocked the AMPH-dependent effect. One possible explanation for these cognitive effects may be that chronic stress counteracts the AMPH effect by blocking anxiety-like or fear components induced by AMPH. Supporting this interpretation is the observation that withdrawal from chronic AMPH has been associated with states of increased anxiety and dysphoria in human addicts and increased anxiety-like behavior in experimental animals (Cancela et al., 2001). In addition, cocaine and AMPH regulate transcript peptide that produces anxiety-like behavior in rodents (Chaki et al., 2003). Therefore, because AMPH treatment was associated with anxiety-like behavior, the possibility that object recognition impairments by this group were due to nonmnemonic components cannot be ruled out in the current data.

Chronic AMPH treatment, with or without stress, elevated CORT levels 5 days after cessation of treatment. In contrast, stress alone did not elevate CORT levels over control levels. Chronic restraint stress, given for 21 days, also did not induce a significant increase in CORT levels in female rats, possibly due to a habituation effect (Bowman et al., 2001). Because current CORT measurements were made immediately following the object recognition test, increases in the AMPH-treated rats may have been due to their heightened anxiogenic-like responses. However, it should also be noted that CORT levels were not associated with performance of the recognition memory task because the stress + AMPH group explored the new object more than the old object whereas the AMPH-treated group did not. Yet, both groups showed similar increases in CORT levels. Consistent with this pattern of results, Wilson et al. (2004) show a disassociation between effects of drugs on anxiety and changes in CORT levels.

Both stress and psychostimulant effects have been linked to increased DA release in the mesolimbic circuit of the brain and the nucleus accumbens in particular (Piazza and Le Moal, 1996). Additionally, estrogen has rapid effects in the caudate nucleus to enhance dopamine release, and it has been suggested that estrogenic-dependent modulation of dopamine in the caudate nucleus might be responsible for the enhanced behavioral response to psychomotor stimulants in females as compared to males (see Becker, 1999 for review). Stress also affects the mesolimbic circuit, and these effects are dependent upon glucocorticoids, because hormone administration increases DA in the Nac while adrenalectomy produces the opposite effect (Piazza and Le Moal, 1996). Current data suggest that stress-regulated mesocortical dopamine circuits may also be affected by chronic psychostimulant administration. Levels of synaptophysin were decreased in the CA1 region of the hippocampus in AMPH- or AMPH + stress-treated groups (see below for discussion). The hippocampus exerts tonic inhibition over CORT release (McEwen, 2000).

Thus, decreases in this synaptic protein may also be involved in AMPH-dependent CORT increases. However, these responses to chronic stress and AMPH need to be further investigated in males because most information on these systems has been obtained in this sex.

Stress alone, in the current paradigm of 21 days of daily restraint sessions, was not associated with any specific behavioral alteration in females. Reports from our laboratories, and others, show that female responses to stress differ from male responses. Female subjects are either not affected or show improvements in several memory tasks following stressors that impair male performance (Conrad et al., 2003; for review, see Luine, 2002). A different time course of sensitivity in males versus females for the change from stress adaptive to maladaptive responses has been proposed, possibly due to the presence of estradiol in females (Rhodes and Frye, 2004; Bowman et al., 2003). The present study extends these observations and shows that female rats may also be able to tolerate/manage two chronic stressors—in this case, restraint and AMPH treatment—without deleterious consequences to some neural functions.

Synaptophysin is a synaptic protein involved in regulation of vesicular exocytosis, specifically in the docking and fusion steps of calcium-dependent vesicular exocytosis (Bergmann et al., 1993; Grabs et al., 1994; Papa and Segal, 1996). In the hippocampus, synaptophysin expression was affected by AMPH, both AMPH treatment alone or in combination with stress resulted in a reduction of synaptophysin expression in the CA1 region (stratum oriens). Frick et al. (2002) reported that estrogen increased synaptophysin expression in the hippocampus of aged female mice, and this increase was correlated with improvements in reference memory in a spatial memory task. We found no changes in synaptophysin expression after chronic stress in this area. Chronic restraint stress induces CA3 dendritic retraction in male rats, but this effect is less robust and occurs in a smaller region in females, suggesting that female rats are less vulnerable to stress-induced neuronal atrophy (Magariños and McEwen, 1995; Galea et al., 1997).

In the DML area of the caudate–putamen, chronic AMPH treatment increased synaptophysin expression compared to controls; however, no significant differences were found between controls and subjects receiving a combination of stress + AMPH. In addition, in the VL area, both chronic AMPH and stress increased synaptophysin expression compared to controls. Again, no significant differences between control and stress + AMPH treatment were found. These results are in agreement with a recent study (Li et al., 2003) showing that chronic AMPH treatment increases spine density in the dorsolateral area of the caudate–putamen. In our study, RICC, as compared to Golgi methods, was used to quantify synaptophysin expression. RICC techniques have been shown to be as sensitive as Golgi methods in detecting dendritic spine changes associated with synaptic reorganization/plasticity (Brake et al., 2001; Hinz et al., 2001). Altogether, these data indicate that the dorsal–lateral area of caudate nucleus is a sensitive area for synaptic reorganization induced by chronic AMPH treatments. The dorsolateral area is considered a predominantly motor area because it receives major projections from the motor cortex (Gerfen, 1992), but it also receives strong input from the basolateral amygdala, an area involved in motivation and fear reactions (Fass et al., 1984). Therefore, the effects of stress and AMPH on synaptophysin expression in the dorsolateral caudaute putamen may have contributed to changes in performance of the objection recognition task. While the role of basal ganglia structures, like the caudate nucleus, in processing of nonspatial recognition memory is currently unclear (Steckler et al., 1998), work of Packard et al. (1994) and Floresco et al., 1997 suggests that basal ganglia structures are important in some memory processing. In the present experiments, AMPH treatment was associated with anxiety-like behaviors, and thus, we cannot rule out the possibility that object recognition impairments were due to nonmnemonic components, such as motivation and locomotion, both functions regulated, at least in part, by the caudate nucleus.

We have shown that repeated psychostimulant administration induces lasting alterations in neuronal pathways involved in learning and locomotor control. Repeated administration of AMPH or cocaine (using a prolonged treatment period with escalating AMPH doses and 1 month withdrawal period) induces increased dendritic branching in the nucleus accumbens and cortex (Robinson and Kolb, 1997). However, another study found decreased synaptophysin expression in the nucleus accumbens after chronic treatment with AMPH (Subramaniam et al., 2001). In the present study, using a different AMPH treatment regime and female subjects, no changes in either the core or shell of the nucleus accumbens were found; however, it remains possible that synaptic changes induced by psychostimulants are sex, area, and treatment-intensity specific. The long-lasting nature of synaptic protein changes evidenced in other models, such us kindling—increased synaptophysin immunoreactivity in hippocampus (Li et al., 2002)—suggests that alterations of synaptophysin expression observed in this study may represent plastic adaptations at caudate/hippocampal levels to chronic AMPH and stress. However, it is not known whether the changes in synaptophysin expression reported here are long lasting or permanent.

In conclusion, we report a specific environment–drug interaction (chronic stress–AMPH) that counteracts some behavioral and plasticity effects of chronic psychostimulant treatment while still potentiating previously reported psychostimulant locomotor effects. Thus, it is possible that implementation of environmental strategies could counteract some psychostimulant abuse effects. Clearly, further investigations are necessary to test this hypothesis. It is also critical to assess these environment–drug interactions in males, the sex which has received greater attention in both stress and psychostimulant models.

Acknowledgements

This work was supported by funds from MIDARP (R24 DA12136), SCORE (SO6 GM60654), RISE (R25 GM60665) grants (V. Luine), and NSF Conference Grant IBN-0344103. V. Bisagno is currently a recipient of a postdoctoral fellowship from Fundación Carolina, Spain. A portion of these results was presented at the 33rd Society for Neuroscience meeting (2003) and at the 66th Annual Meeting of the College on Problems of Drug Dependence (2004).

References

Alexander GE, Crutcher MD, DeLong MR. Basal ganglia–thalamocortical circuits: parallel substrates for motor, oculomotor, "prefrontal" and "limbic" functions. Prog Brain Res 1990;85:119–46.

Badiani A, Cabib S, Puglisi-Allegra S. Chronic stress induces strain-dependent sensitization to the behavioral effects of amphetamine in the mouse. Pharmacol Biochem Behav 1992;43:53–60.

Barr AM, Hoffman CE, Weinber J, Phillips AG. Exposure to repeated, intermittent D-amphetamine induces sensitization of HPA axis to a subsequent stressor. Neuropsychopharmacology 2002;26(3):286–94.

Becker JB. Gender differences in dopaminergic function in striatum and nucleus accumbens. Pharmacol Biochem Behav 1999;64(4):803–12.

Bergmann M, Shuster T, Grabs D, Marqueze-Pouey B, Betz H, Traurig H, et al. Synaptophysin and synaptoporin expression in the developing rat olfactory system. Brain Res Dev Brain Res 1993;74:235–44.

Bergstrom BP, Schertz KE, Weirick T, Nafziger B, Tackacs SA, Lopes KO, et al. Partial, graded losses of dopamine terminals in the rat caudate–putamen: an animal model for the study of compensatory adaptation in preclinical parkinsonism. J Neurosci Methods 2001;106:15–28.

Bisagno V, Ferguson D, Luine VN. Chronic D-amphetamine induces sexually dimorphic effects on locomotion, recognition memory and brain monoamines. Pharmacol Biochem Behav 2003a;74:859–67.

Bisagno V, Bowman RE, Luine VN. Functional aspects of estrogen neuroprotection. Endocrine 2003b;21(1):33–41.

Bowman RE, Zrull MC, Luine VN. Chronic restraint stress enhances radial arm maze performance in female rats. Brain Res 2001;904:279–89.

Bowman RE, Beck KD, Luine VN. Chronic stress effects on memory: sex differences in performance and monoamines. Horm Behav 2003;43:48–59.

Brake WG, Alves SE, Dunlop JC, Lee SJ, Bulloch K, Allen PB, et al. Novel target sites for estrogen action in the dorsal hippocampus: an examination of synaptic proteins. Endocrinology 2001;142(3):1284–9.

Camp DM, Robinson TE. Susceptibility to sensitization: I. Sex differences in the enduring effects of chronic D-amphetamine treatment on locomotion, stereotyped behavior and brain monoamines. Behav Brain Res 1988a;30(1):55–68.

Camp DM, Robinson TE. Susceptibility to sensitization: II. The influence of gonadal hormones on enduring changes in brain monoamines and behavior produced by the repeated administration of D-amphetamine or restraint stress. Behav Brain Res 1988b;30(1):69–88 [Camp DM, Robinson TE. Susceptibility to sensitization. II].

Cancela LM, Basso AM, Martijena ID, Capriles NR, Molina VA. A dopaminergic mechanism is envolved in the anxiogenic-like response induced by chronic amphetamine treatment: a behavioral and neurochemical study. Brain Res 2001;309:179–86.

Chaki S, Kawashima N, Suzuki Y, Shimazaki T, Okuyama S. Cocaine- and amphetamine-regulated transcript peptide produces anxiety-like behavior in rodents. Eur J Pharmacol 2003;464:49–54.

Conrad CD, Grote KA, Hobbs RJ, Ferayorni A. Sex differences in spatial and non-spatial Y-maze performance after chronic stress. Neurobiol Learn Mem 2003;79(1):32–40.

Conrad CD, Jackson JL, Wieczorek L, Baran SE, Harman JS, Wright RL, et al. Acute restraint stress impairs spatial memory in male but not female rats: influence of estrous cycle. Pharmac Biochem Behav 2004;78:569–79; in this issue.

Covington III HE, Miczek KA. Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration "binges". Psychopharmacology 2001;158(4):388–98.

Ennaceur A, Delacour JA. New one-trial test for neurobiological studies of memory in rats: I. Behavioral data. Behav Brain Res 1988;31:47–59.

Fass B, Talbot K, Butcher LL. Evidence that efferents from the basolateral amygdala innervate the dorsolateral neostriatum in rats. Neurosci Lett 1984;44:71–5.

Floresco SB, Seamans JK, Phillips AG. Selective roles for hippocampal, prefrontal cortical and ventral striatal circuits in radial-arm maze tasks with or without a delay. J Neurosci 1997;17:1880–90.

Frick KM, Fernandez SM, Bulinski SC. Estrogen replacement improves spatial reference memory and increases hippocampal synaptophysin in aged female mice. Neuroscience 2002;115(2):547–58.

Galea LAM, McEwen BS, Tapanat P, Deak T, Spencer RL, Dhabhar FS. Sex differences in dendritic atrophy of CA3 neurons in response of chronic restraint stress. Neuroscience 1997;81(3):689–97.

Gerfen CR. The neostriatum mosaic: multiple levels of compartmental organization in the basal ganglia. Annu Rev Neurosci 1992;15:285–320.

Grabs D, Bergmann M, Schuster T, Fox PA, Brich M, Gratz M. Differential expression of synaptophysin and synaptoporin during pre- and postnatal development of the rat hippocampal network. Eur J Neurosci 1994;6:1765–71.

Hinz B, Becher A, Mitter D, Schulze K, Heinemann U, Draguhn A, et al. Activity-dependent changes of the presynaptic synaptophysin–synaptobrevin complex in adult rat brain. Eur J Cell Biol 2001;80:615–9.

Koob GF, Sanna PP, Bloom FE. Neuroscience of addiction. Neuron 1998;21(3):467–76.

Li S, Reinprecht I, Fahnestock M, Racine RJ. Activity dependent changes in synaptophysin immunoreactivity in hippocampus, piriform cortex and entorhinal cortex of the rat. Neuroscience 2002;115:1221–9.

Li Y, Kolb B, Robinson TE. The location of persistent amphetamine-induced changes in the density of dendritic spines on medium spiny neurons in the nucleus accumbens and caudate–putamen. Neuropsychopharmcology 2003;28:1082–5.

Lin D, Bruijnzeel W, Schmidt P, Markou A. Exposure to chronic mild stress alters thresholds for lateral hypothalamic stimulation reward and subsequent responsiveness to amphetamine. Neuroscience 2002;114(4):925–33.

Luine VN. Sex differences in chronic stress effects on memory in rats. Stress 2002;5(3):205–16.

Lynch WJ, Carroll ME. Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. Psychopharmacology (Berl.) 1999;144(1):77–82.

Magariños AM, McEwen BS. Stress-induced atrophy of apical dendrites of hippocampal CA3c neurons: comparison of stressors. Neuroscience 1995;69(1):83–8.

Masliah E, Ferry RD, Alford M, DeTeresa R. Quantitative immunohistochemistry of synaptophysin in human neocortex: an alternative method to estimate density of presynaptic terminals in paraffin sections. J Histochem Cytochem 1990;38:837–44.

McEwen BS. The neurobiology of stress: from serendipity to clinical relevance. Brain Res 2000;15(886(1–2)):172–89.

Packard MG, Cahill L, McGaugh JL. Amygdala modulation of hippocampal-dependent and caudate nucleus-dependent memory processes. Proc Natl Acad Sci U S A 1994;91:8477–81.

Papa M, Segal M. Morphological plasticity in dendritic spines of cultured hippocampal neurons. Neuroscience 1996;71:1005–11.

Piazza PV, Le Moal M. Pathophysiological basis of vulnerability to drug abuse: interaction between stress, glucocorticoids and dopaminergic neurons. Annu Rev Pharmacol Toxicol 1996;36:359–78.

Rashid K, Van der Zee CEEM, Ross GM, Chapman CA, Stanisz J, Riopelle

BS, et al. A nerve growth factor peptide retards seizure development and inhibits neuronal sprouting in a rat model of epilepsy. Proc Natl Acad Sci U S A 1995;2:9495–9.

Rhodes ME, Frye CA. Estrogen has mnemonic-enhancing effects in the inhibitory avoidance task. Pharmac Biochem Behav 2004;78:551–8; in this issue.

Robinson TE, Kolb B. Persistent structural modifications in nucleus accumbens and prefrontal cortex neurons produced by previous experience with amphetamine. J Neurosci 1997;21:8491–7.

Roth ME, Casimir AG, Carroll ME. Influence of estrogen in the acquisition of intravenously self-administered heroin in female rats. Pharmacol Biochem Behav 2002;72(1–2):313–8.

Russo SJ, Festa ED, Fabian SJ, Gazi FM, Kraish M, Jenab S, et al. Gonadal hormones differentially modulate cocaine-induced conditioned place preference in male and female rats. Neuroscience 2003;20: 525–33.

Silva-Gomez AB, Rojas D, Juarez I, Flores G. Decreased dendritic spine density on prefrontal cortical and hippocampal pyramidal neurons in post-weaning social isolation rats. Brain Res 2003;983(1–2):128–36.

Steckler T, Drinkenburg WHIM, Sahgal A, Aggleton JP. Recognition memory in rats—neuroanatomical substrates. Prog Neurobiol 1998;54: 313–32.

Subramaniam S, Marcotte ER, Srivastava LK. Differential changes in synaptic terminal protein expression between nucleus accumbens core and shell in the amphetamine-sensitized rat. Brain Res 2001;901: 175–83.

Wilson MA, Burghardt PR, Ford KA, Wilkinson MT, Primeaux SD. Anxiolytic effects of diazepam and ethanol in two behavioral models: comparison of males and females. Pharmac Biochem Behav 2004;78:445–58; in this issue.

Wood GE, Shors TJ. Stress facilitates classical conditioning in males but impairs conditioning in females through activational influences of ovarian hormones. Proc Natl Acad Sci U S A 1998;95:4066–71.

Woolley C, Gould E, McEwen BS. Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. Brain Res 1990;531:225–31.