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Pharmacology, Biochemistry and Behavior 74 (2003) 859–867
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
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Chronic D-amphetamine induces sexually dimorphic effects on locomotion, recognition memory, and brain monoamines

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Received 30 August 2002; received in revised form 6 December 2002; accepted 17 December 2002

Abstract

While acute and chronic D-amphetamine (AMPH) treatments produce greater scores for locomotor activity in female rats in comparison with male rats, little is known about AMPH-induced gender differences on cognition. The objectives of the present study were to (1) investigate during a withdrawal period following chronic AMPH treatment whether performance of two memory tasks, object recognition (OR) and object placement (OP) are altered, and (2) determine if an AMPH challenge dose after a withdrawal period amplifies previously reported gender differences in locomotor activity and neurochemistry. Sprague–Dawley male and female adult rats were included in a chronic AMPH treatment (10 injections, 1 every other day; males: 3 mg/kg, females 2.6 mg/kg). Locomotor activity was quantified (acute, chronic, and after a 16-day withdrawal period). Neurotransmitter levels in brain areas were evaluated after an AMPH challenge dose on the 16th withdrawal day. During the withdrawal period, OR (2- and 4-h delays) was impaired in AMPH-treated males but they did not show any impairment in OP; AMPH females also showed impairments in OR (only 4-h delay). AMPH females showed more locomotion after acute and chronic treatment but AMPH-induced hyperactivity was comparable for females and males after a challenge dose. Following a challenge dose of AMPH after a withdrawal period, gender differences in dopaminergic and serotonergic neurotransmission in the striatum were found. These gender differences elicited by AMPH in monoaminergic pathways may be related to sex differences on behavioral components involved in locomotion and OR memory.

Keywords: D-Amphetamine; Sexually dimorphic effects; Recognition memory

1. Introduction

Sex steroids exert potent influences on the nervous system during critical developmental periods and into adulthood by organizing and reorganizing the neuronal circuitry involved in neuroendocrine and behavioral functions (Matsumoto, 1991). Gender differences have been reported for anatomic or functional characteristics of several neurotransmitter systems including the dopamine system (Becker, 1999). Gender differences in this system may have a major impact on numerous complex brain functions since dopamine pathways are involved in motor control, reward circuits, sexual behavior, affective state, and cognitive tasks (Camp and Robinson, 1988, Becker and Beer, 1986, Alexander et al., 1990, Kimura, 1996, Koob et al., 1998). There is also a convergence of neural circuits associated with learning/memory and with those responsible for drug addiction (for review see Nestler, 2001). Drugs of addiction activate the mesolimbic dopamine system, which includes connections between basal ganglia and nucleus accumbens and prefrontal cortex (Koob et al., 1998, Alexander et al., 1990). In rodents, the caudate/striatum modulates different types of learning (Viaud and White, 1989; Packard et al., 1994). In humans, the caudate is thought to be involved in complex cognitive functioning and a recent study found that in women, but not men, better cognitive performance was associated with higher dopamine availability in the caudate and putamen (Harper Mozley et al., 2001). Prefrontal cortex also represents one of the main brain areas involved in working memory, specifically visual recognition memory (Ennaceur et al., 1997).

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

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doi:10.1016/S0091-3057(03)00017-0

chronic amphetamine (AMPH) treatments (Camp and Robinson, 1988; for review see Becker, 1999). It has also been reported that female rodents show a variety of different responses to psychostimulants drugs, including increased motivation to self-administration for cocaine and methamphetamine (Lynch and Carroll, 1999; Roth et al., 2002) and enhanced sensitivity to conditioned place preference for cocaine (Quinones-Jenab et al., 2001). It is not clear whether these gender differences in psychomotor/reward aspects of behavior also extend to other brain functions and, more importantly, to cognitive functions. The aim of the first part of the paper was to verify AMPH effects and sex differences on locomotion (acute and chronic treatments). We also investigated the functional effects on working memory of this AMPH treatment in female and male rats during a withdrawal period from chronic AMPH treatment. We used the one trial object-recognition (OR) paradigm developed by Ennaceur and Delacour (1988), as well as a modification of this test used to evaluate spatial memory, the Object Placement (OP) test (Ennaceur et al., 1997). These tests assess both exploration and working memory (spatial and nonspatial visual memory) by using delays between the sample trial and the recognition trial. Other authors have reported sex differences in neurochemistry following a withdrawal period (Camp and Robinson, 1988), but there is no data regarding the effect of an AMPH challenge after a withdrawal period. Therefore, we further exposed the rats to an AMPH challenge dose after a 16-day withdrawal period and quantified monoamine levels in brain areas in order to determine whether a challenge dose with AMPH intensifies gender differences in the withdrawal period.

2. Materials and methods

2.1. Animals

Intact adult male and female Sprague–Dawley rats (females: 190–200 g, males 240–270 g) were obtained from Harlan, single housed under a 14:10 light/dark cycle (lights on 7:00 a.m.) with water and food ad libitum. Experiments started 2 weeks after arrival.

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

For chronic AMPH treatment, the experimental animals received an intraperitoneal (ip) injection of D-amphetamine sulfate (AMPH) 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 ($N=8$ both males and females). All injections were given in the home cage. Male rats ($N=10$) received 3 mg/kg AMPH on all treatment days as well as on withdrawal ("withdrawal" refers to the absence of AMPH) challenge day, 16 days after the last AMPH injection. Since testicular hormones accelerate AMPH metabolism, resulting in lower AMPH brain levels in intact males than in females given the same systemic dose (Becker et al., 1982); we used a schedule in which female AMPH-treated rats ($N=10$) received 2.6 mg/kg in the first 9 injections and 2.0 mg/k in 10th injection and in the withdrawal challenge day. A lower dose for females (2.6 mg/kg) than males (3 mg/kg) equalizes drug effects, according to Camp and Robinson (1988). Moreover, these authors suggested the use of an even lower dose for female rats in a chronic treatment/challenge dose in order to have a more conservative test; they reported that even using a lower dose (2 mg/kg) in the 10th injection, females showed augmented locomotor and stereotypic responses to AMPH. See schedule illustrated graphically in Fig. 1.

2.3. Amphetamine-induced locomotion

Locomotor activity was quantified automatically over 5-min intervals for 2.5 h on day 1 (first AMPH injection) and day 10 (last AMPH injection). We used separate photocells chambers (San Diego Instruments, San Diego, CA) operated simultaneously. Animals were habituated to the test apparatus for 30 min prior to drug administration. Testing session started immediately after the injection. Injections #2 to #9

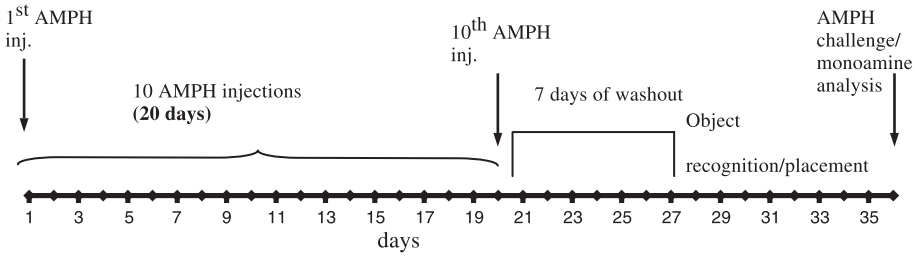

Fig. 1. Graphic representation of the experimental protocol used to asses amphetamine-withdrawal effect on behavioral and neurochemical variables. Experimental groups received 10 AMPH injections during 20 days. Locomotion was measured in days #1 and #10. After a 7-day washout period open field, OR and OP were performed (total days after last AMPH injection: 16 days), then drug-pretreated animals received a challenge AMPH dose. Animals were sacrificed (for brain monoamine detection) following locomotion measurements.

were administered in the home cages; after the injections, the animals were left undisturbed in their cages. We also quantified ambulation in response to a challenge dose of AMPH after 16 days of withdrawal during 2 h.

2.4. Object-recognition and object-placement tests

The behavioral tests started with open-field trials in order to test general activity and acclimate subjects to the testing field. The floor was marked off into 15 equal (20.5 cm) squares (5 × 3). For subsequent trials that had objects on the field, the area was shortened to nine squares (3 × 3).

In the open-field trial (7 days after the last AMPH injection), subjects' activities (sector visits, rears, wall climbs) were recorded for 6 min. OR memory trials were conducted. OR sessions consisted of two 3-min trials: a sample trial (T1) and a recognition trial (T2), each separated by an intertrial interval. The total time each subject interacted with each of the objects was recorded. In these trials, two identical objects were placed equidistant from the north corners during T1, but for T2, one of them was switched with a novel object. Additional trials were added where the OR task was adjusted from a nonspatial memory test to a spatial memory test: the OP test (Ennaceur et al., 1997). In this version of the task, instead of replacing one of the sample objects with a novel object, one of the sample objects is moved to a new location on the field for the test trial. We tested the subjects using 1-, 2-, and 4-h intertrial delays.

Intertrial delays for OR and OP were chosen based on previous studies in our laboratory which reported that, even though performance became less consistent with long intertrial delays (especially long delays in OP for female rats), rats could discriminate novel objects or novel locations of objects in this range (Bisagno et al., 2002, Beck and Luine, 2002). The objects used were a variety of soda cans and plastic bottles. We used the same objects for females and males, and both the objects and the field were carefully cleaned between trials. The order in which the tests were performed (for all the subjects) was open field, OR tests, and then OP tests in a sequential order. These tests 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 within each separate delay session 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 are expressed as total exploration time in T1 (seconds, mean ± S.E.M.) and time spent with the old and novel objects in T2 (seconds, mean ± S.E.M.).

2.5. Neurochemical analyses

On day 16 after the last AMPH or saline injection, subjects were singly placed in the locomotor test cages and given a challenge injection (injected with AMPH or saline). Two hours later, they were taken to a separate room and sacrificed by decapitation (without anesthesia). Their brains were quickly removed and immediately placed in dry ice. The brains were subsequently stored at −70 °C until HPLC analysis. First, a caudal slice was made to remove the frontal lobe; gross 1-mm slices were made from the frontal lobe and used for frontal cortex sampling. The remaining brain was sliced (300 um) using a microtome cryostat. The frozen striatum, substancia nigra, ventral tegmental area (VTA), and nucleus accumbens were sampled by tissue punch (6–8 punches per region). These samples were centrifuged with a 60-μl sodium acetate buffer (containing internal standards of alpha-methyl-dopamine and homoserine). The supernatant was used to assess levels of monoamines using high-performance liquid chromatography (Waters 2960 Alliance and Waters 717 autosampler with 590 pump) with electrochemical detection (ESA Coulochem II) (see Beck and Luine, 2002 for details). Monoamine levels were assessed with a 40-μl injection of the supernatant using a C-18 reverse-phase column for separation (Waters Nova-Pak). The protein pellet was resuspended and quantified using the Bradford method (Beck and Luine, 1999). Sample runs for monoamines averaged 18–25 min. Peak heights were assessed and quantified using Millenium Chromatography software (Waters). The internal standard and protein in each sample were used to calculate total sample amounts. Tissue levels for monoamines are expressed as pg/μg protein.

2.6. Data analysis

Locomotor activity (including data from AMPH injection #1 and #10) was analyzed using ANOVA with repeated measures (sex × treatment × injection #). LSD test was used as a post hoc test when appropriate. Locomotor activity during the AMPH withdrawal challenge was analyzed using ANOVA (sex × treatment); LSD was used as a post hoc test when appropriate. For OR/Placement ANOVAs with repeated measures (sex × treatment × delay) were utilized to test differences in exploration time during T1; ANOVAs with repeated measures were used to test differences in time spent with the objects (old and new) during T2 (sex × treatment × object × delay), paired t tests on each group tested whether time spent with the old object (or location) was less than time spent with the new object (or location). Neurochemical data was analyzed using ANOVA (sex × treatment), LSD was used as a post hoc test when appropriate. For all statistics, a significance level of $P < .05$ was established.

3. Results

3.1. Locomotion

The effect of repeated, intermittent AMPH treatment on locomotion is reported in Fig. 2. Locomotion was quan-

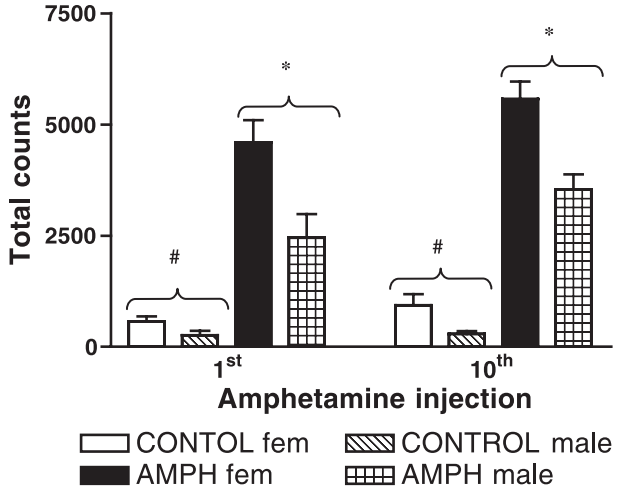

Fig. 2. Effect of repeated, intermittent AMPH treatment on locomotion. Total activity counts cumulated over a 2.5-h session following AMPH administration (males 3 mg/kg, females 2.6 and 2 mg/kg in injection #10). Females showed more total counts than males, $^{\#}P<.01$: AMPH groups had more total counts than control groups, $P<.01$: a sex × treatment effect. $^{*}P<.05$: female AMPH had more locomotor activity than male AMPH-treated rats. There was an injection # effect (1 or 10): in injection #10 animals showed more counts than in injection #1, and an injection # × sex effect, $P<0.05$: both female and males had more counts in injection #10. Bars represent the mean ± S.E.M.

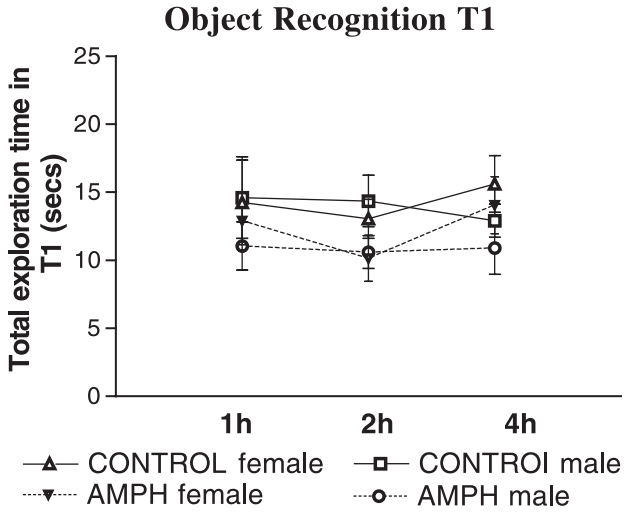

Fig. 4. Total exploration time in the sample trial (T1) of the OR task in each delay during AMPH withdrawal period. Data are mean ± S.E.M.

tified in control and AMPH-treated rats after the 1st and the 10th injections. Repeated measures ANOVA (sex × treatment × injection #) revealed a significant effect of sex. [$F(1,32)=16.32$, $P<.001$]: females had more total counts than males, a treatment effect [$F(1,32)=116.86$, $P<.000001$]: AMPH groups had more total counts than control groups, a sex × treatment effect [$F(1,32)=6.01$, $P<.02$]: AMPH-treated females had more locomotion than male AMPH-treated rats, injection # effect (1 or 10) [$F(1,32)=9.65$, $P<.003$]: injection #10 showed more counts than injection #1, and a injection # × sex effect [$F(1,32)=4.95$, $P<.04$]: both female and males had more counts in injection #10. No other statistical interactions were found.

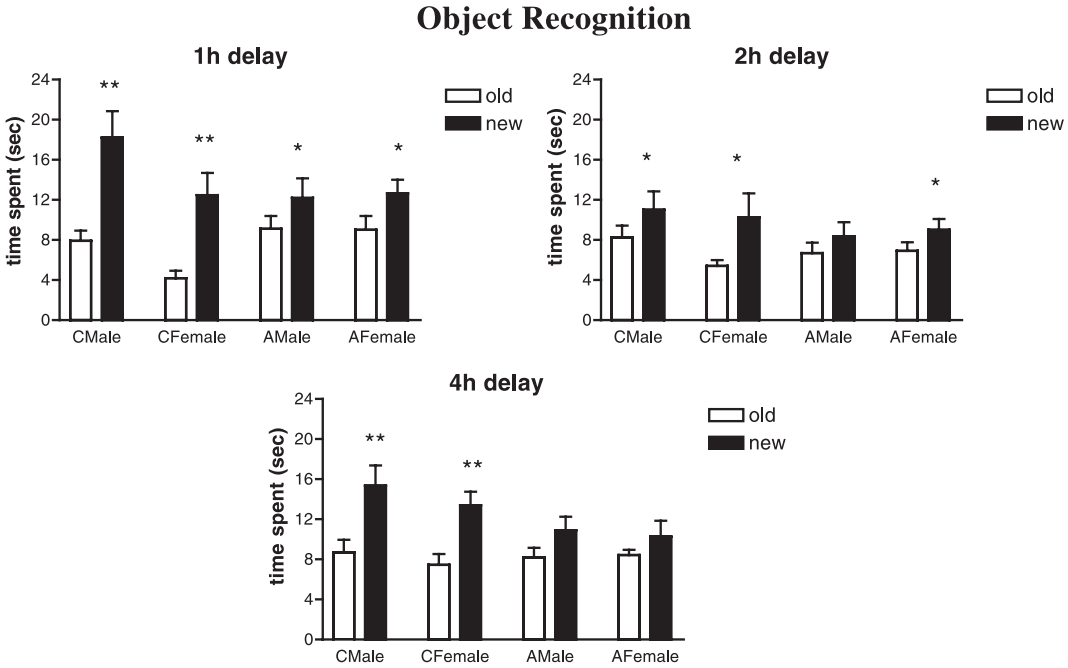

Fig. 3. OR memory during AMPH withdrawal. Bars represent the mean time (± S.E.M.) exploring the old and the new object in the recognition trial (T2). ANOVA showed a significant difference in time spent with the objects (old and new). $^{**}P<.01$, $^{*}P<.05$ (paired t test) within each group.

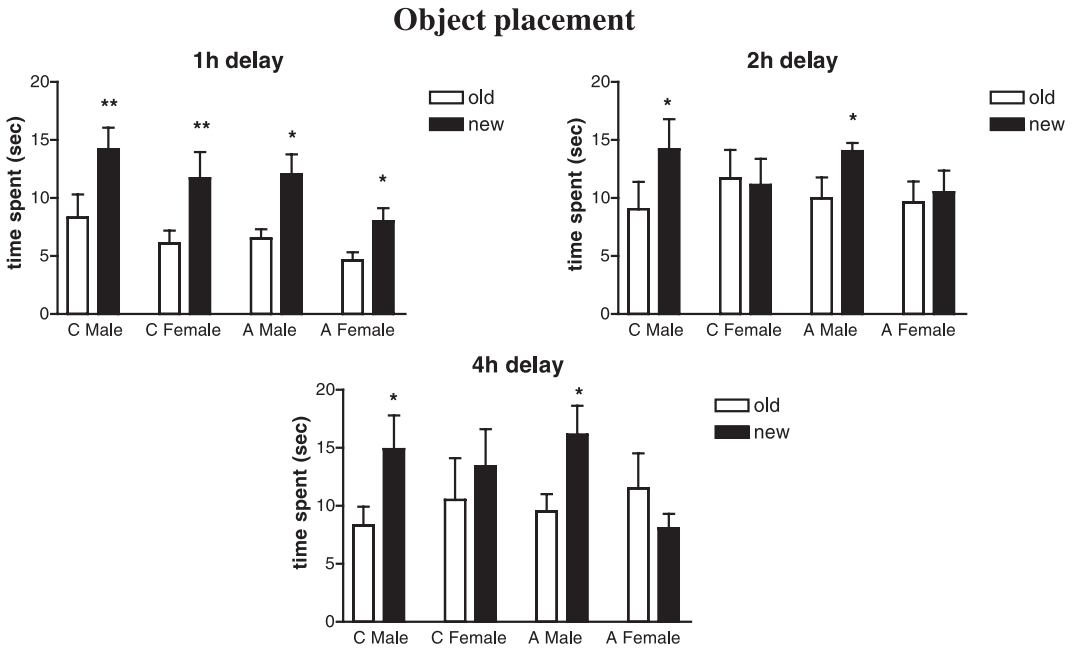

Fig. 5. OP memory after AMPH administration. Bars represent the mean time (±S.E.M.) exploring the old and the new object in the recognition trial (T2) during AMPH withdrawal. ANOVA showed a significant difference in time spent at the locations (old and new) and a significant interaction, sex × object, effect. $**P<.01$, $*P<.05$ (paired t test) within each group.

3.2. Object recognition and placement tests

3.2.1. Object recognition

Seven days following the last (10th) AMPH injection cognitive testing of the groups began. No drug injections were given during the behavioral testing. Prior to the OR test, exploratory behavior in an open field was evaluated. Using a two-way ANOVA, we found a sex difference only in wall climbs: females groups had more wall climbs than male rats [$F(1,32)=26.57$, $P<.0001$] (data not shown). The other exploratory parameters were not different between groups or sexes.

For total exploration time in sample trial (T1) (Fig. 4), repeated measures ANOVA did not show any significant differences between the groups. In addition, it should be noted that AMPH treatment did not modify T1 exploration times. In the recognition trial (T2), ANOVA showed a significant difference in time spent with the objects (old and new) [$F(1,225)=88.41$, $P<.000001$], a delay effect [$F(2,225)=4.05$, $P<.05$], and a significant interaction, delay × drug, effect [$F(1,225)=20.15$, $P<.0001$]. Compar-

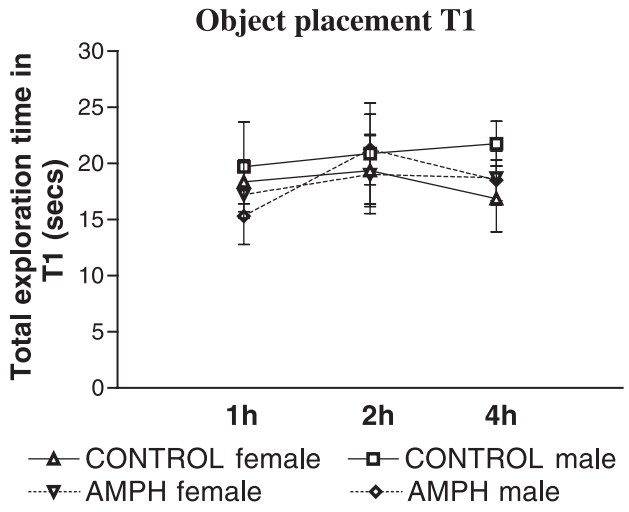

Fig. 6. Total exploration time in the sample trial (T1) of the OP task in each delay during AMPH withdrawal period. Data are mean±S.E.M.

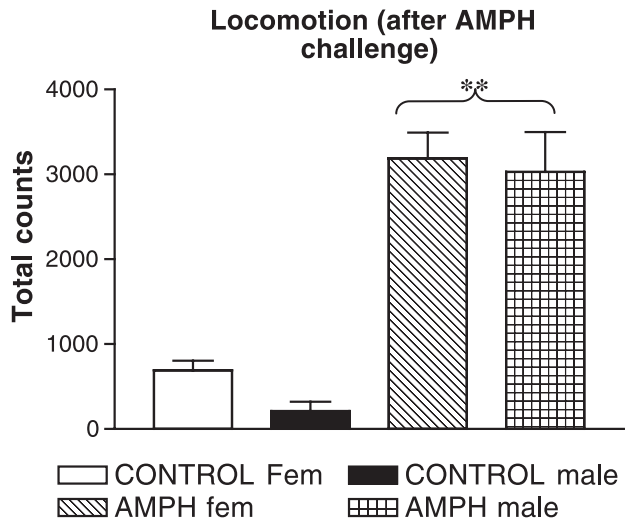

Fig. 7. Effect of an AMPH challenge dose (males 3 mg/kg, females 2 mg/kg) after a 16-day withdrawal period on locomotion. Two-way ANOVA (sex × treatment) showed a treatment effect, AMPH groups had more counts than control groups. No statistical interaction (sex × treatment) was found. $**P<.01$ compared with control groups.

ison of the time spent with the old and the new objects within groups indicated that the control groups (both females and males) explored the new object longer than the familiar one in the 1-, 2- and 4-h delay (paired Student's t test, $P<.01$, $P<.05$ and $P<.01$, respectively) (Fig. 3). AMPH-treated males explored the new object longer only in the 1-h delay, while AMPH-treated females explored the new object longer in the 1- and 2-h delays (paired Student's t test, $P<.01$, $P<.05$ and $P<.01$, respectively). With a 4-h intertrial delay, neither AMPH-treated groups explored the new object longer than the old object.

3.2.2. Object placement

Regarding the total exploration time in sample trial (T1), repeated measures ANOVA did not show any significant differences between the groups (Fig. 6).

In the recognition trial (T2), ANOVA showed a significant difference in time spent with the objects (old and new) [$F(1,225)=88.41$, $P<.01$], and a significant interaction, sex × object, effect [$F(1,225)=20.15$, $P<.05$] (Fig. 5). Comparison of the time spent at the old and the new locations within groups indicated that male groups (both control and AMPH-treated rats) explored the object in the new location longer than the familiar location in the 1-, 2- and 4-h delay trials (paired Student's t test, $P<.01$ and $P<.05$, respectively). In contrast to males, females (control and AMPH) could only discriminate between old and new locations at the 1-h delay; thus, it was not possible to further evaluate AMPH effect on OP in females (Fig. 5).

3.3. Amph challenge after withdrawal period

3.3.1. Locomotion

Sixteen days after the 10th AMPH injection (approximately 1 h after the conclusion of the OP test) an AMPH challenge dose was given. Two-way ANOVA (sex × treat-treatment) showed a treatment effect [$F(1,29)=60.37$, $P<.00001$], AMPH groups had more counts than control groups. However, this challenge AMPH injection did not induce sex or sex × treatment effects in locomotor activity (Fig. 7).

Table 1
Effect of an AMPH challenge after a 16-day withdrawal period on brain monoamine levels

	DA	DOPAC	HVA	HVA/DA	5-HT	5-HIAA	5-HIAA/5HT	NE
Striatum								
F Con	153.2±8	23.4±2[a]	4.14±0.25	0.03±0.001	3.11±0.22[a]	1.95±0.19	0.64±0.07	nd
F AMP	173.6±14[b]	23.8±3[a]	4.96±0.32[b]	0.03±0.004	3.9±0.36[a,b]	2.31±0.20[b]	0.59±0.04	nd
M Con	163.1±12	30.1±3	4.13±0.18	0.02±0.001	2.7±0.24	1.65±0.04	0.63±0.06	nd
M AMP	169.3±5[b]	28.7±2	4.9±0.32[b]	0.03±0.002	3.4±0.17[b]	1.83±0.07[b]	0.53±0.01	nd
Nucleus accumbens								
F Con	122.8±14.3	18.5±30	9.4±0.66	0.07±0.004	9.4±0.06	6.3±0.44	0.67±0.03	12.2±4[a]
F AMP	158.4±32	17.4±2.8	12.9±3.3	0.08±0.01	12.7±2.3	10.7±2.80	0.88±0.22	14.8±3.7[a]
M Con	139.5±10.8	20±1.30	10.7±0.8	0.07±0.006	12±1.30	7.25±0.55	0.63±0.06	17.3±3.2
M AMP	165.2±24.7	19.7±2.30	11.01±2.2	0.06±0.004	14.2±2.10	8.22±1.35	0.57±0.02	30.4±6.7
Prefrontal cortex								
F Con	0.65±0.1	0.42±0.08	0.28±0.09	0.40±0.07	2.07±0.26	1.40±0.11	0.71±0.06	nd
F AMP	0.60±0.08	0.62±0.21	0.27±0.07	0.57±0.24	2.50±0.17	1.40±0.14	0.58±0.05	nd
M Con	0.50±0.06	0.30±0.08	0.16±0.02	0.34±0.05	2.01±0.26	1.45±0.17	0.76±0.11	nd
M AMP	0.76±0.18	0.64±0.26	0.21±0.04	0.30±0.04	2.27±0.19	1.40±0.19	0.62±0.07	nd
Substancia nigra								
F Con	13.7±2.8	2.17±0.38	2.40±0.40	0.19±0.03	22.2±3.70	4.3±0.40	0.21±0.03	5.20±1.70
F AMP	13.8±1.2	2.20±0.50	2.7±0.50	0.20±0.01	34±12.10	7.35±1.50[b]	0.14±0.04[b]	3.50±1.70
M Con	14.2±2.9	2.80±0.80	3.10±0.90	0.23±0.04	25.3±3.30	3.80±0.40	0.15±0.01	10.20±3.70
M AMP	14.8±1.2	2.17±0.30	2.80±0.30	0.19±0.02	37±3.90	5.05±0.50[b]	0.13±0.04[b]	7.40±2.50
VTA								
F Con	9.60±1.90	4.28±0.65	2.81±1.12	0.28±0.07[a]	6.30±1	4.75±0.78	0.82±0.16	6.15±1.10
F AMP	4.88±0.85	7.02±4.59	1.37±0.22	0.29±0.02[a]	6.03±1	5.06±0.92	0.84±0.07	5.10±0.61
M Con	8.45±2.81	3.5±1.16	1.60±0.53	0.20±0.02	5.27±1.27	3.12±0.90	0.57±0.03	7.02±2.07
M AMP	8.55±1.24	3.85±0.67	1.90±0.32	0.22±0.01	5.98±0.41	4.54±0.62	0.74±0.07	6.71±0.53

In striatum, two-way ANOVA indicated a significant treatment effect on DA, AMPH-treated groups had higher levels of DA than control groups and 5-HIAA. In DOPAC and 5-HT, a sex effect was found: females had lower levels of DOPAC and higher levels of 5-HT. In nucleus accumbens, females had lower levels of NE than males. In substancia nigra, AMPH groups had higher levels of 5-HIAA and lower 5-HIAA/5HT ratio than control groups. In VTA, only a marginal sex effect was found in HVA/DA ratio: females had higher HVA/DA ratio than male rats. No significant effects of sex or treatment were found in prefrontal cortex on neurotransmitter levels.

[a] Indicates a sex effect.

[b] Indicates a treatment effect.

3.3.2. Neurochemistry

Immediately following locomotor activity testing (2 h after the AMPH challenge injection), subjects were sacrificed and brains removed for neurotransmitter measurements. As shown in Table 1, the AMPH challenge dose, after a 16-day withdrawal period, altered neurotransmitter and metabolites in several brain areas. In striatum, sex and treatment effects were noted on several neurochemicals. Two-way ANOVA indicated a significant treatment effect on DA, AMPH-injected groups had higher levels of DA than control groups [$F(1,23) = 6.22$, $P < .05$] as well as on HVA levels [$F(1,23) = 9.74$, $P < .004$], 5-HT levels [$F(1,23) = 14.67$, $P < .001$], and 5-HIAA [$F(1,23) = 11.43$, $P < .01$]. In DOPAC and 5-HT, a sex effect was found: females had lower levels of DOPAC and higher levels of 5-HT [$F(1,26) = 5.44$, $P < .05$ and $F(1,26) = 4.38$, $P < .05$, respectively] irrespective of drug treatment. In nucleus accumbens, differences in NE were found. Females had lower levels of NE than males [sex effect, $F(1,28) = 4.32$, $P < .05$]. In substancia nigra, AMPH groups had higher levels of 5-HIAA [treatment effect, $F(1,27) = 7.25$, $P < .05$] and lower 5-HIAA/5-HT ratio than control groups [$F(1,27) = 8.53$, $P < .01$]. In VTA, only a marginal sex effect was found in HVA/DA ratio: females had higher HVA/DA ratio than male rats [$F(1,21) = 4.3$, $P = .05$]. No significant effects of sex or treatment were found in prefrontal cortex on neurotransmitter levels.

4. Discussion

The primary goal of the present study was to investigate if the gender differences in locomotor and stereotypical behavior induced by AMPH administration extend to functional cognitive performance in the OR test (nonspatial visual memory) and OP test (spatial visual memory). These tests are useful to assess both object exploration and working memory using different delay periods between the sample and the recognition trial. These different intertrial delays produce different degrees of difficulty. Thus, it is possible to assess either enhancements or impairments in performance by varying intertrial delay length; with shorter delays, the animals can discriminate more easily the novel object than with longer delays (Ennaceur et al., 1997). In the AMPH withdrawal period (7–16 days), both AMPH male and females rats showed impairments in discriminating the novel object compared with control groups. Other authors have reported that intermittent AMPH treatment alters the acquisition of operant conditioning, but only male rats were investigated (Taylor and Jentsch, 2001). Our results expand the literature by showing even though AMPH withdrawal has a deleterious effect on OR memory on both sexes, treated females showed impairments in this task only with the longer intertrial delay (4 h); however, males showed impairments with short and long delays (2 and 4 h). While performance was impaired by treatment, it is unclear whether these impairments are related to attentional deficits, difficulty to initiate actions, or specific deficits in storage/retrieval mechanisms. A different pattern of result was observed in the OP test. We found that treated males did not show impairments in any of the three delays studied, 1-, 2- or 4-h delay. Thus, in males, chronic AMPH treatment did not impair OP performance. Female groups (both control and AMPH-treated subjects) only could discriminate the novel location of a familiar object in the 1-h delay. Recent findings from our laboratory also showed that female rats showed impairments in OP tasks with intertrial delays longer than 1 h (Beck and Luine, 2002). Thus, we were unable to fully evaluate the females in this task. Male rats have less difficulties discriminating novel location of objects (Beck and Luine, 2002, Bisagno et al., 2002). These results suggest that the effects of AMPH withdrawal on cognition may also depend on task demand and/or a difference in cognitive processing and their underlying neurophysiological mechanisms. Reports from Mostafa and Ennaceur (2000) suggest that spatial tasks may be more demanding than nonspatial ones because an object can be identified and discriminated from several features (shape, color, etc) while a spatial location offers fewer cues.

Consistent with previous reports (Camp and Robinson, 1988), an acute AMPH injection induced gender differences in locomotion, female treated rats had more total counts. Females had higher locomotion and activity levels than males even following a saline injection. Thus, the possibility that AMPH amplifies a different baseline level cannot be completely ruled out. Interestingly, during AMPH withdrawal, we found that both genders showed comparable locomotion and stereotypy scores after an AMPH challenge dose. However, AMPH-treated females exhibited more activity after the 10th AMPH injection compared to the AMPH challenge dose, whereas males showed the same amount of activity following the 10th and the challenge injection. These data might suggest that there is a gender difference in the expression of AMPH-induced hyperactivity following a withdrawal period.

Neurochemical analysis showed sex and treatment differences in dopaminergic brain areas. In striatum, we found that a challenge dose increased DA and 5-HT neurotransmission and in substantia nigra only 5-HT was increased. In nucleus accumbens, norepinephrine showed a sex difference, females had lower levels than males. We did not find significant changes in dopamine neurotransmission in prefrontal cortex following AMPH treatment. Paulson et al. (1991) reported (in female rats, no gender comparisons) that an AMPH challenge after a withdrawal period (28 days) increased DA levels in striatum and elevated norepinephrine in the nucleus accumbens; no differences were reported in prefrontal cortex.

Camp and Robinson (1988), using the same AMPH doses for a longer period but without a AMPH challenge stimulus, found that previous AMPH exposure enhanced striatal DOPAC/DA and HVA/DA ratios in treated females

but not in treated males. Our study shows that a challenge dose of AMPH after a withdrawal period caused not only drug effects but also gender differences on dopaminergic and serotonergic neurotransmission in the striatum and on norepinephrine content in the nucleus accumbens. These gender differences in monoaminergic pathways may be related to sex differences in cognitive variables involved in locomotion and OR memory.

General explorative activity did not appear affected by chronic AMPH, spontaneous behavior in the open field was not statistically different between treated and control animals (following a 7-day withdrawal period). A sex difference was found in wall climbs: females were more active than males in this variable. Similarly, the total exploration time in the sample trials for OR and OP were not different in AMPH-treated rats. Therefore, it can be assumed that after AMPH withdrawal, treated rats did not show motor disabilities or substantial changes in their exploratory activity. Using a more prolonged schedule of escalating AMPH doses, other authors showed that AMPH-treated rats had behavioral depression for at least 1 week, which was only evident in nocturnal activity (Paulson et al., 1991). Even though we used a constant low dose of AMPH with a duration of 1/3 of the Robinson's study, the possibility that treated animals had a depression-like condition cannot be completely ruled out.

In humans, infants with prenatal exposure to cocaine and/or amphetamine have lower scores in a visual recognition memory task (Struthers and Hansen, 1992), and adult chronic amphetamine users show impairment on a test of pattern recognition memory (Ornstein et al., 2000) as well as other visual memory tasks (McKetin and Mattick, 1997). In a recent study, Strakowski et al. (2001) showed progressive changes in subjective responses (euphoria) following repeated amphetamine administration to healthy humans, and this effect was greater in women; no cognitive tasks were performed. Other studies suggest that acute amphetamine administration improves cognitive performance by modifying selective attention, but again, no gender differences were evaluated (McKetin and Mattick, 1997; Servan-Schreiber et al., 1998).

Mechanisms underlying amphetamine effects on learning and memory are largely unexplored. Acute AMPH stimulates dopamine release and induces an activation of the mesocorticolimbic system. Repeated administration and withdrawal causes transient adaptations in neurochemistry and neuroanatomy of dopamine pathways (Paulson et al., 1991, Robinson and Kolb, 1997). In addition, it has been suggested that some cognitive changes associated with AMPH abuse may be related to altered dopaminergic modulation of the prefrontal cortex and striatum, and possibly of the "functional loops" operating between the two (Alexander et al., 1986; Rogers et al., 1999). Acute AMPH and withdrawal might differentially affect these dopaminergic circuits and then influence behaviors in a distinctive way.

Another possibility is that AMPH can mediate its effect on cognition indirectly, acting as a stressor (Antelman et al., 1980). In this line of evidence, Cancela et al. (2001) showed that chronic treatment with AMPH induced an anxiogenic effect in male rats on the elevated plus maze. Corticosterone and ACTH are increased after an acute AMPH injection (Swerdlow et al., 1993) and psychostimulant sensitization depends, in part, on stress-induced corticosterone secretion (Deroche et al., 1995). More over, other authors report that stress cross-reacts with AMPH (Camp and Robinson, 1988). Additionally, it has been established that males and females show different behavioral changes following stress (Heinsbroek et al., 1990), and more interestingly, there are gender differences in the cognitive effects of stress (Luine et al., 2001): like AMPH, stress generally impairs male performance more than female performance.

In conclusion, we have found that in a drug-free period following AMPH administration, male rats show impaired performance of a visual recognition task. Females, show less impairments in the same task following AMPH. Spatial memory in the males was not affected by prior AMPH exposure. As reported by others and also confirmed here, AMPH causes greater increases in locomotion in female as compared to male rats which may reflect the effect of AMPH in different baseline activity level between female and male rats. While mechanisms underlying these effects of AMPH are unclear, effects on DA may be important since DA circuits contribute to all these behaviors and showed differences between males and females. Thus, taken together, these results suggest that sex and/or gonadal hormones may be important contributors to psychostimulant effects on behavior. Clearly, further clinical and basic studies are necessary to understand the complex relationships between psychostimulant drugs, sex, stress, and dopaminergic circuits in regulating behavior especially higher order functions like learning and memory.

Acknowledgements

This work was supported by funds from MIDARP (R24 DA12136, NIDA), SCORE (SO6 GM60654), and RISE (R25 GM60665) grants. We wish to thank R. Kesraj, A. Lalmansingh, and L. Jacome for helping with behavioral observations. A portion of these results was presented at the 64th Annual Scientific Meeting of the College on Drug Dependence (2002), Quebec City, Canada.

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