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Estrogen Effects on the Hyperactivity Induced by (+)-MDMA and Cocaine in Female Rats

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This study compared the effects of estrogen (E) on the hyperactivity induced by (+)-3,4-methylenedioxymethamphetamine (MDMA) with E effects of cocaine-evoked hyperactivity in female rats. Sprague-Dawley rats were ovariectomized (OVX); half of them received a 17β-estradiol (E_2) implant (OVX + E). Three weeks later, rats received saline, (+)-MDMA (1, 2, or 4 mg/kg) or cocaine (5, 10, or 20 mg/kg), and locomotor activity was monitored. OVX + E rats exhibited greater locomotor hyperactivity in response to both psychostimulants than did OVX rats. The enhanced response to cocaine appeared within 5 min following drug injection whereas the enhanced response to (+)-MDMA was delayed for approximately 30 min. The differential effects of E on hyperactivity may be due to the unique profiles of DA and 5-HT in response to (+)-MDMA and cocaine.

Sex differences in the behavioral response to psychostimulants, such as cocaine, have been found in both humans and experimental animals. For example, women cocaine users demonstrated different patterns of abuse than men (Griffin, Weiss, Mirin, & Lange, 1989). The self-reported "nervous" response elicited by intranasal cocaine is greater and the duration of the "high" is longer in women than in men (Kosten et al., 1996). In rats, females demonstrate a substantially greater locomotor hyperactivity in response to cocaine than do males (Glick, Hinds, & Shapiro. 1983; Sell, Scalzitti, Thomas, & Cunningham. 2000; van Haaren & Meyer, 1991). In addition, female rats develop self-administration of cocaine more readily than males (Lynch & Carroll, 1999). Evidence suggests that differences in metabolism between genders do not appear to account for the enhanced sensitivity of female rats to cocaine (Bowman et al., 1999; Thompson, Shuster, Casey. & Kanel, 1984). Although multiple mechanisms could underlie these sex differences, ovarian hormones are thought to play an important role. For example, the sex differences in response to

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cocaine emerge after puberty (Bowman & Kuhn, 1996), and hyperactivity induced by cocaine in female rats has been shown to he greater during proestrus and estrus than in diestrus animals (Sell et al., 2000). In addition, we have shown that cocaine-induced hyperlocomotion in OVX female rats was enhanced by chronic estrogen (E) in the presence or absence of progesterone (P: Sell et al., 2000). Thus, these studies indicate that E is a major contributor to the sex differences in the behavioral response to cocaine.

A derivative of amphetamine, 3,4-methylenedioxymethamphetamine (MDMA, ecstasy), has become a popular recreational drug in youth culture. The positive subjective effects of MDMA include feelings of mental stimulation, emotional warmth, closeness, and empathy for others, and decreased anxiety (Greer & Tolbert, 1986; Vollenweider, Gamma. Liechti, & Huber, 1998). However, there have been an increasing number of reports of severe acute toxicity and death following ingestion of the drug (Green, Cross, & Goodwin, 1995). When MDMA is given to rats, hyperactivity as a component of the serotonin syndrome and hyperthermia rapidly follow (Colado, Murray. & Green, 1993; Dafters, 1994). Repeated or high-dose administration (> 20 mg/kg) of MDMA to rats is neurotoxic to a subpopulation of 5-hydroxytryptamine (5-HT)-containing axons that project to the forebrain (Green et al., 1995). Although we are unaware of other data in the literature addressing gender differences in the locomotor response to MDMA, a recent report from our laboratory has shown that female rats express a significantly greater degree of hyperactivity in response to 4 mg/kg of (+)-MDMA compared with males (Bubar, Thomas. & Cunningham, 2001). To date, little has been reported regarding the mechanisms underlying the sex differences in the behavioral response to MDMA. We hypothesized that analogous to its role in the effects of cocaine, E plays a role in the sex differences in the hyperactivity induced by (+)-MDMA in rats.

MDMA and cocaine are both substrates for the reuptake transporters for 5-HT, dopamine (DA), and norepinephrine (Slikker et al., 1989). Although cocaine inhibits reuptake at these sites, MDMA inhibits monoamine oxidase (Leonardt & Azmttia, 1994) and increases release of monoamine neurotransmitters by reversal of the transporter (Rudnick & Wall, 1992) Despite the fact that both drugs potentiate efflux of DA and 5-HT, a different profile of enhanced DA and 5-HT neurotransmission is seen with MDMA relative to cocaine. For example, whereas an immediate increase in 5-HT efflux is seen following MDMA injection, rises in extracellular DA levels are delayed (White, Duffy, & Kalivas, 1994), in contrast to cocaine, which increases extracellular DA and 5-HT concentrations simultaneously (Broderick & Phelix, 1997). Furthermore, MDMA-induced DA release in vivo was attenuated by a selective serotonin reuptake inhibitor fluoxetine (Koch & Galloway, 1997). These data suggest that MDMA-evoked DA release is at least partially dependent on functional 5-HT neurons. Thus, whereas the behavioral effects of cocaine are thought to be predominantly mediated by enhanced DA neurotransmission, a unique contribution of 5-HT has been proposed to mediate the neuropharmacology of MDMA (Bankson & Cunningham, 2001, 2002; Callaway, Johnson, Gold. Nichols, & Geyer, 1991; McCreary, Bankson, & Cunningham. 1999). Therefore, it was of interest to compare the effects of E on hyperactivity induced by (+)-MDMA with those induced by cocaine in female rats.

In the present study, the effects

of E on the dose-response relationship of (+)-MDMA (1, 2, and 4 mg/kg ip) and cocaine (5, 10,20 mg/kg ip) to evoke hyperactivity in OVX female rats was explored. The (+)-isomer of MDMA was chosen for study because of its greater potency in eliciting hyperactivity compared with the (-)-isomer (Paulus & Geyer, 1992), whereas these doses of (+)-MDMA were chosen because of their limited potential to result in neurotoxicity in rats (O'Shea. Gronados, Esteban. Colado, & Green, 1998) and the likelihood that at low doses the primary action of (+)-MDMA is as an indirect 5-HT agonist (Bankson & Cunningham, 2001, 2002; Callaway, Rempel, Peng, & Geyer, 1992; Koch & Galloway, 1997).

Method

Subjects

Adult female (180-200 g) Sprague-Dawley (virus antibody-free) rats were obtained from Harlan (Houston. TX) and housed in our animal facility for 1 week prior to the start of experimentation. The rats were housed 4 per cage with food and water available ad libitum (except during experimental sessions) and maintained at a constant temperature (21-23 degrees C and humidity (45%-50%) with lights on 7 a.m. to 7 p.m. All experimental protocols were carried out in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Institutes of Health, 1986) and with approval by the Institutional Animal Care and Use Committee.

OVX and Estrogen Implant Surgery

Adult female Sprague-Dawley rats (200-220 g., n = 8/group, 16 groups total) were anesthetized with a cocktail (1 ml/kg body weight, intramuscular; im) delivering 43 mg/kg of ketamine, 8.6 mg/kg of xylazine, and 1.5 mg/kg of acepromazine in 0.9% NaCI. Surgical bilateral ovariectomy was performed by a dorsal incision. Half of the OVX rats received 4 mm l7□-estradiol (E2) implants (OVX + E) subcutaneously at the nape of the neck as described previously (Sell et al., 2000). In this type of hormone administration, the surface area of the implants determines the dose of the hormone delivered. As Hope, Bruns, and Thotoas (l992) and others (Bridges, 1984) have shown, the 4-mm implant used in these experiments produces an E2 level (40-50 pg/mI) approximating the peak circulating level of E2 achieved in proestrous during the normal estrous cycle in the adult female rats (Siflith, Freeman, & Neil). 1975). Experiments were conducted 3 weeks after surgery and implant placement. During this 3-week period, rats with the same hormone treatment were housed together.

Behavior Measurement

Locomotor activity was monitored and quantified with a modified open field activity system under low light conditions (San Diego Instruments, San Diego, CA) between 11 a.m. and 1 p.m. Eight separate chambers were available for monitoring locomotor activity. Thus we were able to test each drug dose on rats from each cage, assuring that group responses were not due to anomalies with regard to test day or time. Each activity monitor, housed within a sound- and light-attenuating enclosure. consisted of a 40 cm x 40 cm x 40 cm clear plexiglas box within a 4 x 4 matrix of photobeams adjusted to 5 cm above the cage floor to measure horizontal activity. Each monitor was equipped with another horizontal row of 16 photobeams that was located 11 cm above the 4 x 4 photobeam matrix to measure vertical activity. The

control software (PhotobeamActivity Software, PAS-F Version 5-18-95, San Diego Instruments; monitoring system: MedAssociates, St. Albans, VT) counted and stored total horizontal as well as vertical beam interruptions in 5-min bins. A video camera (low-light. adjustable aperture) and video monitor provided the observer with the ability to score rats for the presence of particular behaviors.

A qualitative measure of expression of rodent behaviors was recorded during the automated measurement of activity using a modification of the technique developed by Kelley (1998). At 5,15, 30, 60, 90, and 120 min after the drug injection. each rat was observed for 1 min for expression of behaviors. which included sleeping or inactive, sniffing, ambulation, head movements, and rearing. The total score (range 0-6) of these five behaviors was summed and presented as a total observational score for each rat.

Experimental Design

Experiment I: Effects of E2 on dose-response to (+)-MDMA in OVX rats. Three weeks after the surgery, OVX (n = 8/group, 4 groups total) and OVX + E rats (n = 8/group, 4 groups total) were habituated to the activity chamber for 3 hr/day for 2 days before the start of the experiment. Locomotor activity was recorded during habituation on each day. On the day of testing, rats were placed in the activity chamber and locomotor activity was monitored for 60 mm of habituation. Subsequently. each rat received one challenge injection of saline (1 ml/kg ip) or (1, 2, or 4 mg/kg ip), and activity was monitored for an additional 120 min with concurrent behavioral scoring.

Experiment 2: Effects of E2 on dose-response to cocaine in OVX rats. Three weeks after the surgery, OVX (n = 8/group. 4 groups total) and OVX + E rats (n = 8/group. 4 groups total) were habituated to the activity chamber for 3 hr/day for 2 days before the start of the experiment. Locomotor activity was recorded during the habituation on each day. On the day of testing, rats were placed in the activity chamber and locomotor activity was monitored for 60 mm of habituation. Subsequently, each rat received one challenge injection of saline (1 ml/kg ip) or cocaine (5, 10, or 20 mg/kg ip) and activity was monitored for an additional 120 min with concurrent behavioral scoring.

Drugs

Cocaine hydrochloride or (+)-MDMA (National Institute on Drug Abuse, Research Triangle Park, NC) was dissolved in physiological saline (0.9% NaCI [wt/vol) and administered intraperitoneally such that a volume of 1 ml/kg delivered the desired dose. 17□-estradiol (Sigma, St. Louis, MO) was administered in crystalline form sealed into silastic tubing as described earlier.

Statistical Analysis

Data collected in automated activity monitors for the entire 120-min session are presented as mean *(±SEM)* total horizontal or vertical activity counts for the session. A two-way analysis of variance (ANOVA) was used to assess the effects of hormone treatment, dose [(+)-MDMA or cocaine], and a Hormone Treatment X Dose interaction. Individual mean values were compared using Duncan's multiple-range test. A two-wav ANOVA was used to assess the effects of hormone treatment, dose [(+)-MDMA or cocaine], and a Hormone Treatment x Dose interaction on the total observation scores in OVX rats. A one-way ANOVA was used to detect the effects of E on spontaneous locomotor activity in OVX

rats during the habituation. All statistical analyses were conducted with an experimentwise error rate (alpha) of .05 (SAS for Windows. Version 6.12).

Results

Effects of E2 on Spontaneous Activity in OVX Rats

On Day 1 F(l, 31) = 17.99, p = .00l, and Day 3 of habituation, F(1,31) = 29.22, p = .001, E2-treated rats exhibited significantly more horizontal locomotor activity during the first 60 min in the chamber compared with OVX control rats (Figure 1). Compared with Day 1, OVX and OVX + E rats both showed less horizontal locomotor activity on Day 3, and the Day 1 to Day 3 decrease in horizontal activity appeared to be greater in OVX rats (42%) than in OVX + E rats (25%), although the difference was not statistically significant *(p* = .06). Similar effects of E2 were found for vertical activity on Day 1, *F 1,* 31) = 10.64, p = .0018 and Day 3, *F*(l, 31) = 13.59, p = *.0005,* habituation in OVX rats (Figure 2). Compared with Day 1, on Day 3. OVX and OVX + E rats both showed less vertical activity, and the Day 1 to Day 3 decrease in vertical activity appeared to be greater in OVX rats (48%) than in OVX + F rats (31%), although the difference was not statistically significant.

Experiment 1: Effects of E2 on Dose-Response to (+)-MDMA in OVX Rats

A dose-response relationship for hyperactivity induced by saline or (+)-MDMA (1, 2, and 4 mg/kg) was established using eight independent groups of OVX and OVX + F rats. Figure 3 illustrates the time course of horizontal hyperactivity evoked by (+)-MDMA; the accumulated activity throughout the 120-min test period for each group is summarized in the insert bar plot. A main effect of hormone treatment, *F*(1, 56) = 11.62, p = .0012, and dose, *F*(3, 56) = 48.95, p = .0001. was observed for total horizontal activity. E2-treated rats showed significantly higher horizontal locomotor activity compared with OVX rats after the highest dose (4 mg/kg) of (+)-MDMA *F(*l,56) = 23.22, p = 0001.,

Similar effects of E2 were found for vertical hyperactivity induced by (+)-MDMA in OVX rats (Figure 4). A main effect of hormone treatment, F(1, 56) = 23.80. p = .0001; dose, F(3,56) = 23.09, p = .001; and Dose X Hormone Treatment, F(3,56 = 11.63, p = .0001, was observed for total vertical hyperactivity. E2 significantly increased vertical hyperactivity after 4 mg/kg of (+)-MDMA in OVX rats, *F(*1,56) = l8.28. p = .0001.

All the results for total horizontal and vertical hyperactivity induced by (+)-MDMA are summarized in Figure 5 for comparison.

Observational scores indicated that behaviors (sleeping or inactive, sniffing, ambulation, head movement. and rearing) induced by (+)-MDMA were not different between OVX rats and hormone-treated OVX rats, *F(*1, 56) = 9.71. p = .0618; Figure 6). Thus, it is unlikely that such small differences in expression of observed behaviors in response 10 (+)-MDMA contributed to the large differences in hyperactivity between OVX and OVX + rats as measured in automated chambers.

Experiment 2: Effects of E2 on Dose-Response to Cocaine in OVX Rats

A dose-response relationship for hyperactivity induced by cocaine (5, 10, and

20 mg/kg) was established using eight independent groups of OVX and OVX + E rats. Figure 7 illustrates the time course of horizontal hyperactivity evoked by cocaine; the accumulated activity throughout the 120-mm test period for each group is summarized in the insert bar plot. A main effect of hormone treatment, F(1,56) = 15.42. p = .002; dose, F(3,56) = 51.53, p = .0001; and Dose x Hormone Treatment interaction, F(3,56) = 3.29, p = .0272, was observed for total horizontal activity. Preplanned comparisons indicate that E2-treated rats showed significantly higher horizontal activity after 10 and 20 mg/kg of cocaine compared with that of DYX control rats, $F(1,56) = 20.35$. p = .0001.

Similar effects of E2 were found for vertical hyperactivity induced by cocaine in OVX rats (Figure 8). A main effect of hormone treatment, F(l, 56) = 31.95, p = .0001; dose. F(3, 56)
= 28.32, p = .0001; and Dose x Hormone Treatment interaction. F(3,56) = 8.51, p = .0001. was observed for total vertical hyperactivity. E2 significantly increased vertical hyperactivity induced by 10 or 20 mg/kg of cocaine in OVX rats, F(1,56) = 20.35.p = .0001.

All the results of total horizontal and vertical hyperactivity induced by cocaine are summarized in Figure 9 for comparison.

Observational scores indicated that behaviors (sleeping or inactive, sniffing, ambulation, head movement, and rearing) induced by cocaine were not different between OVX rats and hormone-treated OVX animals, F(l, 56) = 2.74, p = .1033 (Figure 10). Thus, there was no indication that expression of observed behaviors in response to cocaine contributed to the differences in hyperactivity between OVX and OVX + E rats as measured in automated chambers.

Discussion

The present study demonstrated that the same chronic regimen of E2 shown to enhance hyperactivity induced by a low dose of cocaine (Sell et al., 2000) also enhances the hyperactivity induced by (+)-MDMA in OVX female rats. In addition, a similar shift in the dose-effect curve for cocaine was also demonstrated. Con-comitant with the enhanced response to (+)-MDMA and cocaine, E2 significantly enhanced spontaneous locomotor activity in OVX rats compared with OVX alone.

To address the hypothesis that E plays a role in the hyperactivity evoked by (+)-MDMA in rats. our approach was to establish a chronic, steady level of E2 in OVX female rats using hormone-filled silastic implants. In this experiment. there was no attempt to mimic any natural endocrine state, but rather, the goal was to determine whether the presence or absence of circulating E2 could modulate the behavioral response to psychostimulants. It should be emphasized that although the serum concentration of E2 (40-50 pg/ml) established in this model is within the physiological range, a rat is not normally exposed to this peak hormone level for more than 1 day at a time (proestrus) except during pregnancy (Smith et al.. 1975). In the present experiments, these levels were maintained at a steady state for 3 weeks. The decision to focus the present experiments on OVX and E-replaced animals was based on our earlier finding that cocaine-induced locomotor hyperactivity varies across the estrous cycle in female rats, with the response being greatest during proestrus and estrus when estrogen levels are highest, and the subsequent finding that of the ovarian

steroids, E appeared to exert the major effect on this parameter (Sell et al., 2000).

Although the experiments presented here were designed to study the hyperactivity evoked by (+)MDMA and cocaine, we also recorded the spontaneous activity of animals during the habituation paradigm. E2 significantly enhanced spontaneous horizontal and vertical locomotor activity in OVX female rats. These data are consistent with earlier studies of sex differences in spontaneous activity (Hitchcock, 1925) and observations that intrahypothalamic implants of E2 enhanced wheel-running activity in OVX rats (Colvin & Sawyer. 1969). This E2-evoked regulation of activity in novel (possibly stressful) environments may be related to the significantly higher peak of adrenocorticotrophin and corticosterone levels in response to stress during proestrus (which corresponds to the peak level of E2) compared with the estrus and diestrus phases of the estrous cycle (Viau & Meaney, 1991). Thus, E2 may play a facilitatory role in the control of the hypothalamic-pituitary-adrenal axis, which in turn may contribute to the higher basal levels of activity in OVX rats (Viau & Meaney, 1991)

Our findings that E2 enhanced the behavioral response to (+)-MDMA in OVX rats are consistent with the existing body of literature concerning sex differences in response to amphetamine, a parent compound of (+)-MDMA. For example, female rats have been shown to have higher responses than males to amphetamine in terms of hyperactivity (Savageau & Beatty, 1981), rotational behavior, and sensitization (Robinson, Becker, & Presty 1982).

Both amphetamine-evoked hyperactivity and DA release vary with the estrous cycle as E2 and *p* levels change (Becker & Cha 1989), and OVX attenuates amphetamine-induced behavior (Camp, Becker, & Robinson, 1986) and DA release (Becker & Ramirez, 1981). Thus, these results suggest that E2-enhanced DA neurotransmission may be involved in the hyperactivity induced by (+)-NIDMA in female rats.

In a comparative study, the effects of E2 on the dose-response relationship for locomotor hyperactivity induced by cocaine were also established here. Our data indicate that E2 enhances the sensitivity to cocaine by shifting the dose-response curve in OVX rats. The enhanced sensitivity to cocaine in E-treated rats confirms previous studies of sex differences in response to cocaine (Bowman & Kuhn, 1996; Glick et al., 1983; van Hauren & Meser, 1991) and the role of E in the locomotor response to cocaine in female rats (Sell et al., 2000).

One striking observation from the present study is the difference between the time courses of the hyperactivity induced by (+)-MDMA and cocaine. Following cocaine injection. both OVX and OVX + E rats exhibited hyperactivity within 5 mm. After a short interval, hyperactivity declined over the remainder of the 120-min test period. In contrast, (+)-MDMA (4 mg/kg) evoked only a slight increase in locomotor activity in both OVX and OVX + E rats during the first 20 min following injection. Then, between 20 and 40 mm following (+)-MDMA injection, both groups exhibited hyperactivity that was more pronounced in OVX + E than OVX rats. The differences in the pharmacokinetic properties between (+)-MDMA and cocaine may account at least in part for the differential temporal profile of effects. However, the delayed time course for (+)-MDMA-induced hyperactivity corresponds to the delayed appearance of DA release that occurs following direct

application of MDMA into nucleus accumbens in the rat; in contrast to an immediate increase in 5-HT, 20-30 mm elapsed prior to the increase in extracellular levels of DA (White et al., 1994). On the other hand, cocaine increased extracellular DA and 5-HT concentrations immediately and simultaneously (Broderick & Phelix, 1997). Thus, these results suggest that the interaction between E2 and psychostimulants is determined by the unique profiles of DA and 5-HT produced subsequent to administration of a specific drug, in this case (+)-MDMA or cocaine. Furthermore, the findings are consistent with the possibility that E may play a more important role in modifying DA neurotransmission than 5-HT signaling per se.

Compared with horizontal activity, vertical hyperactivity was exquisitely sensitive to the effects of E such that E pretreatment resulted in expression of rearing at 4 mg/kg (+)-MDMA, a dose that did not evoke increased rearing in OVX animals. These results suggest that E2 regulates substrates that play a more prominent role in vertical rearing activity than in horizontal activity. For example, 5-HT2C receptors are thought to be involved in the rearing activity induced by psychostimulants (Bankson & Cunningham, 2001, 2002). However, no change (Bankson & Cunningham, 2001; MeCreary et al., 1999) or suppression in rearing (Callaway et al., 1991, 1992) are the predominant observations following MDMA administration in male rats. Furthermore, administration of a low dose of (+)-MDMA following the blockade of 5-HT2C receptors with SB206553 (Bankson & Cunningham, 2002) or SB242084 in male rats (Herin & Cunningham, 2001) elicits a dramatic expression of rearing, suggesting that 5-HT released by (+)-MDMA results in an indirect stimulation of 5-HT2C receptors to suppress rearing. Thus, in future studies, specific pharmacological manipulations can be designed to investigate the sex-based differential regulation of 5-HT2C receptors and the role of this receptor in the vertical rearing activity evoked by psychostimulants.

One possible mechanism underlying E effects on the hyperactivity evoked by psychostimulants is that E may regulate gene expression of the receptors and/or reuptake transporters within DA and 5-HT systems through which (+)-MDMA and cocaine exert their effects. Other data emerging from our laboratory (Zhou, Cunningham, & Thomas. 2002) suggest that 3-week E2 implant treatment in OVX rats regulates the mRNA levels for D1-like (D1) and D2-like (D2S, D2L, D3) DA receptors and 5-HT2C receptor in several limbic brain areas. Future studies correlating these changes in mRNA with the localization of altered expression of the corresponding proteins will help elucidate the specific receptors and transporters that are involved in the sex differences in the response to psychostimulants.

In conclusion, the present study demonstrates that E enhances the hyperactivity induced by (+)-MDMA. E effects on the time course of locomotor activity evoked by (+)-MDMA and cocaine are different. E may modulate this response via its regulation of gene expression of important components within DA and 5-HT systems in the brain. These results may provide new insights into abuse liability and potential therapeutic strategies for drug dependence in women.

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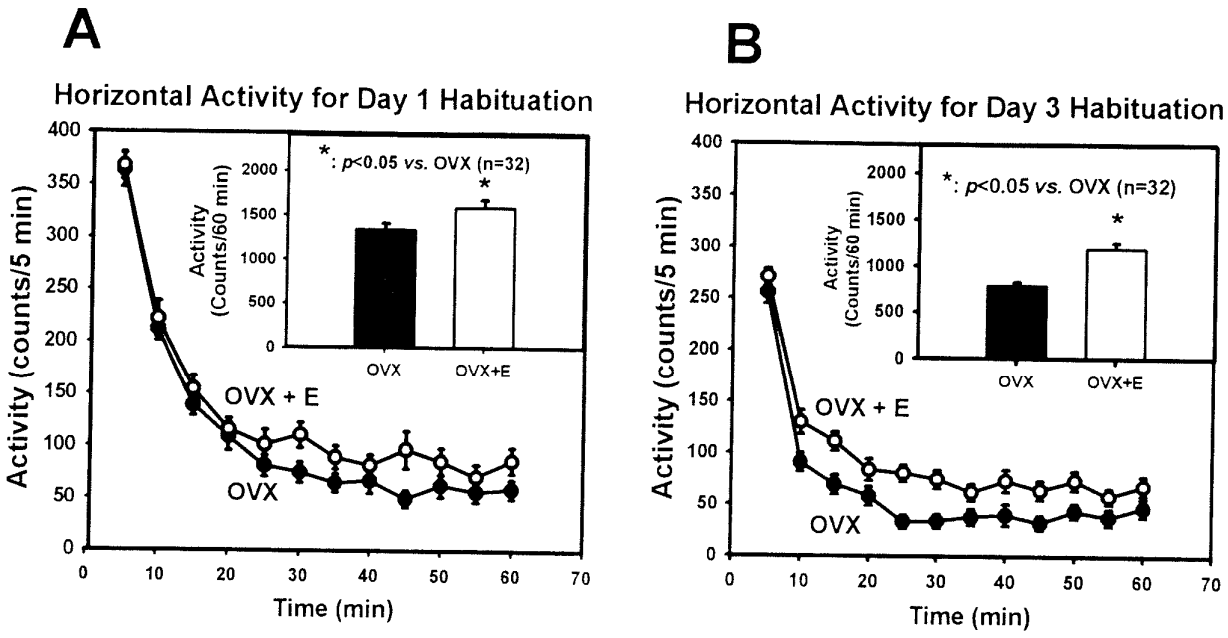

Figure 1. Effects of 17β-estradiol on spontaneous horizontal locomotor activity in ovariectomized (OVX) rats. Horizontal activity was measured in 5-min time epochs for 60 min during the habituation session on Day 1 (A) and Day 3 (B) in OVX (closed bars) and OVX + estrogen (E) rats (open bars; n = 32/group). Mean (±*SEM*) total activity counts for the entire 60-min session are shown for each group as an insert bar plot (* $p < .05$ vs. OVX).

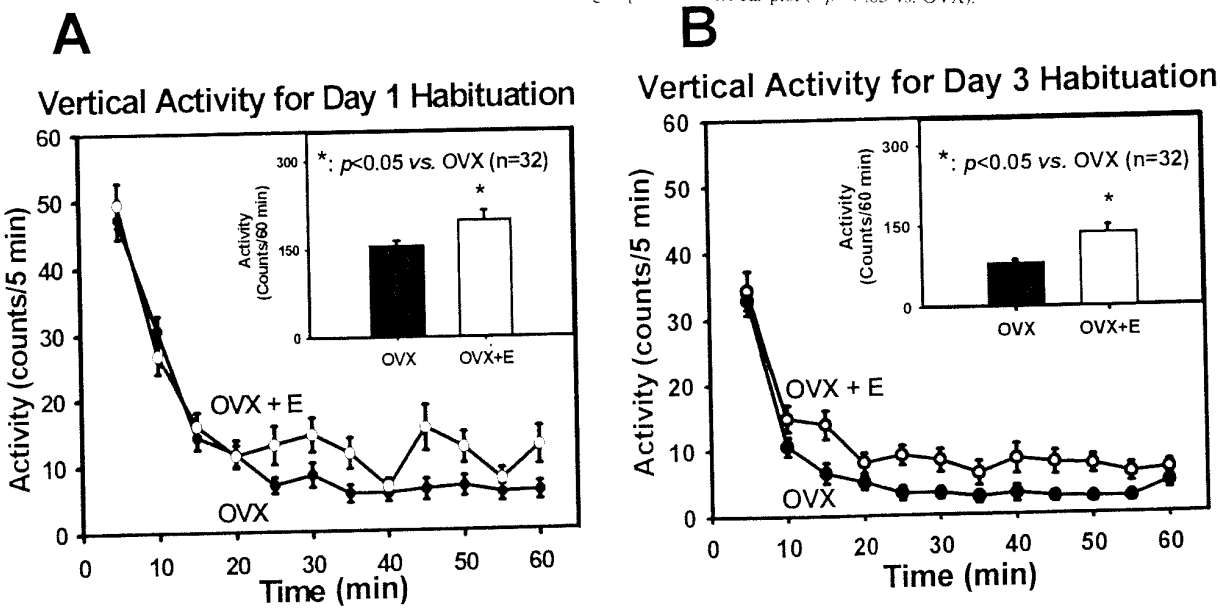

Figure 2. Effects of 17β-estradiol on spontaneous vertical locomotor activity in ovariectomized (OVX) rats. Vertical activity was measured in 5-min epochs for 60 min during the habituation on Day 1 (A) and Day 3 (B) in OVX (closed bars) and OVX + estrogen (E) rats (open bars; n = 32/group). Mean (±*SEM*) total activity counts for the entire 60-min session are shown for each group as an insert bar plot (* $p < .05$ vs. OVX).

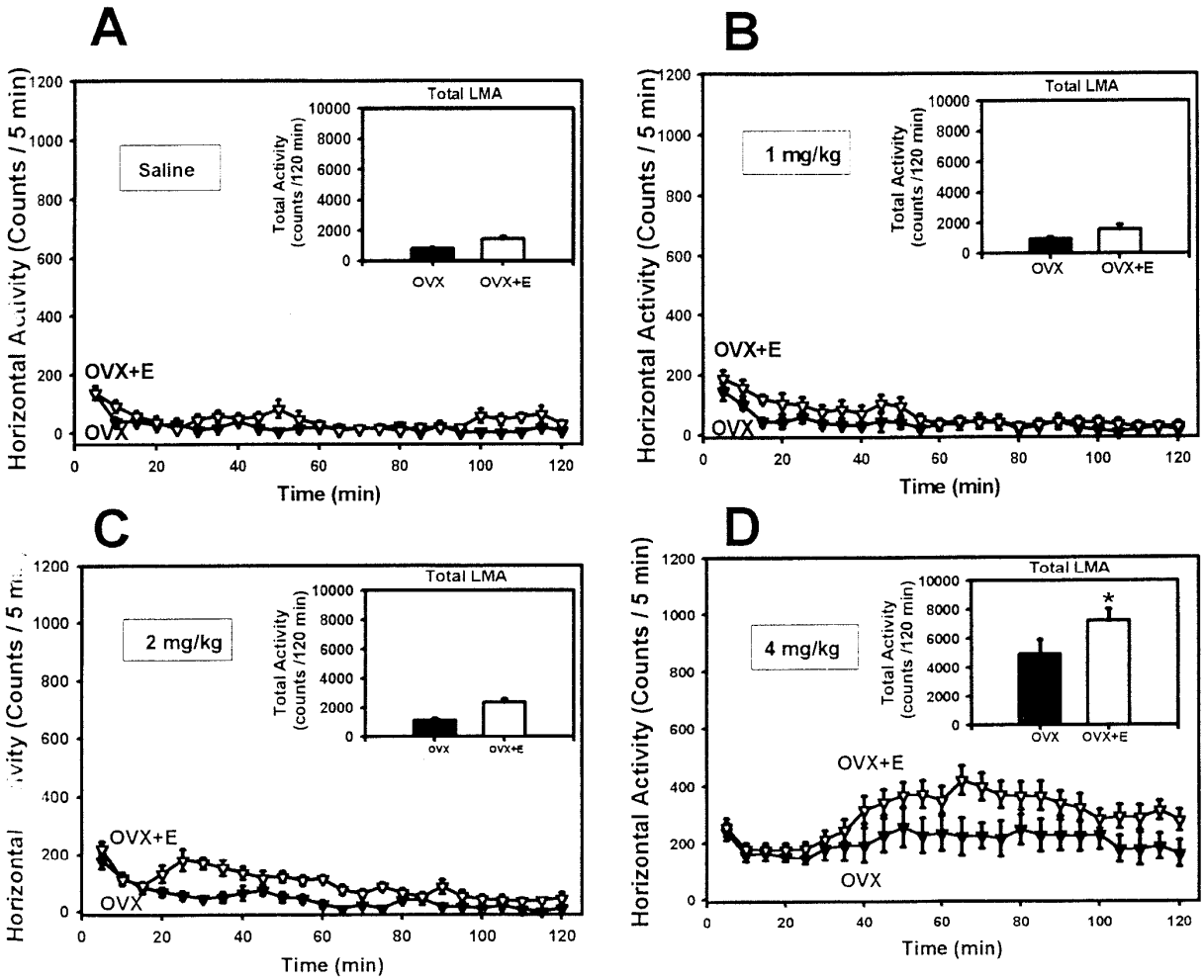

Figure 3. Effects of 17β-estradiol on (+)-3,4-methylenedioxymethamphetamine (MDMA)-evoked horizontal locomotor activity (LMA) in ovariectomized (OVX) rats. The time course of mean (±*SEM*) horizontal activity counts/5 min for the 120-min session is shown for OVX (closed bars) and OVX + estrogen (E) rats (open bars; n = 8/group, 8 groups) after administration of saline (A) or 1 mg/kg (B), 2 mg/kg (C), or 4 mg/kg (D) MDMA. Mean (±*SEM*) total activity counts for the entire 120-min session are shown for each group as an insert bar plot (* $p < .05$ vs. OVX).

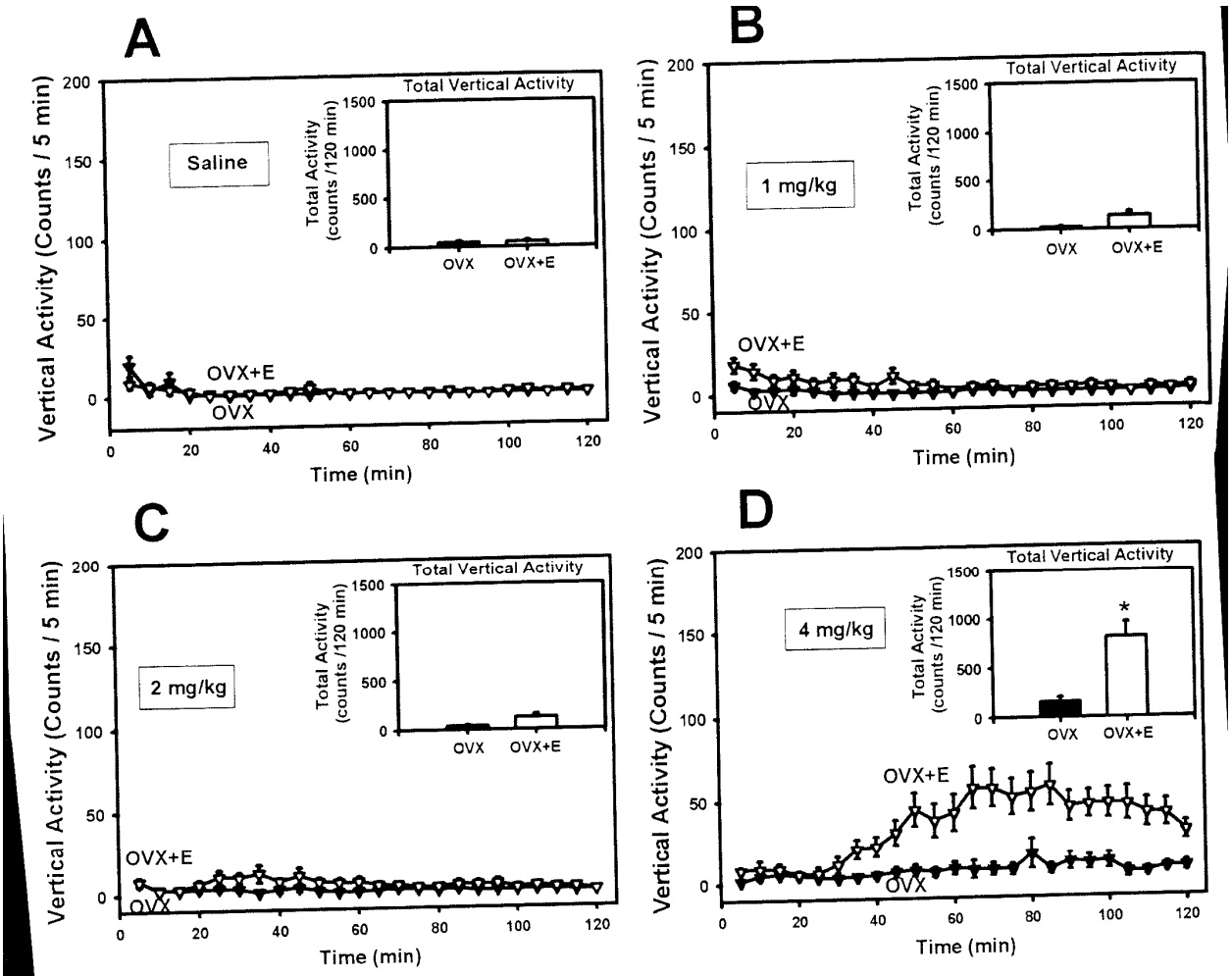

Figure 4. Effects of 17β-estradiol on (+)-3,4-methylenedioxymethamphetamine (MDMA)-evoked vertical locomotor activity of ovariectomized (OVX) rats. The time course of mean (±*SEM*) vertical activity counts/5 min for the 120-min session is shown for OVX (closed bars) and OVX + estrogen (E) rats (open bars; n = 8/group, 8 groups) after administration of saline (A) or 1 mg/kg (B), 2 mg/kg (C), or 4 mg/kg (D) MDMA. Mean (±*SEM*) total activity counts for the entire 120-min session are shown for each group as an insert bar plot (* $p <$ .05 vs. OVX).

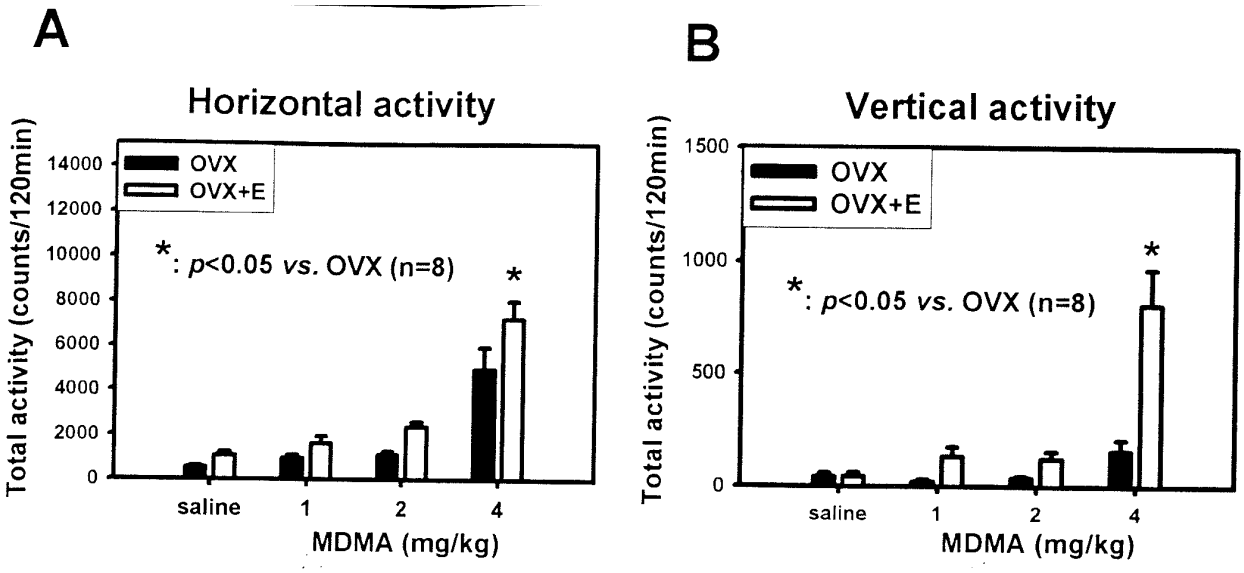

Figure 5. Summary data for 17β-estradiol effects on the dose–response for locomotor activity induced by (+)-3,4-methylenedioxymethamphetamine (MDMA) in ovariectomized (OVX) rats. Mean (±*SEM*) total horizontal (A) and vertical (B) locomotor activity for the 120-min session are shown for OVX (closed bars) and OVX + estrogen (E) rats (open bars; n = 8/group, 16 groups) after administration of saline or 1, 2, or 4 mg/kg (+)-MDMA (* $p < .05$ vs. OVX).

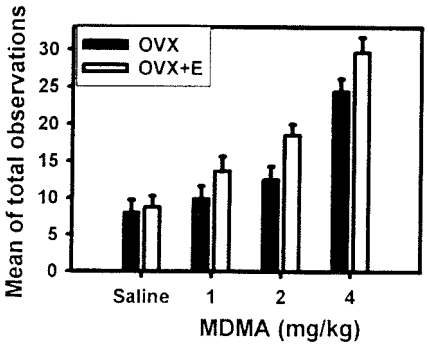

Figure 6. Effects of 17β-estradiol on observed behaviors induced by (+)-3,4-methylenedioxymethamphetamine (MDMA) in ovariectomized (OVX) rats. Total (±*SEM*) scores of observations for the 120-min session are shown for OVX (closed bars) and OVX + estrogen (E) rats (open bars; n = 8/group, 8 groups) after administration of saline or 1, 2, or 4 mg/kg (+)-MDMA.

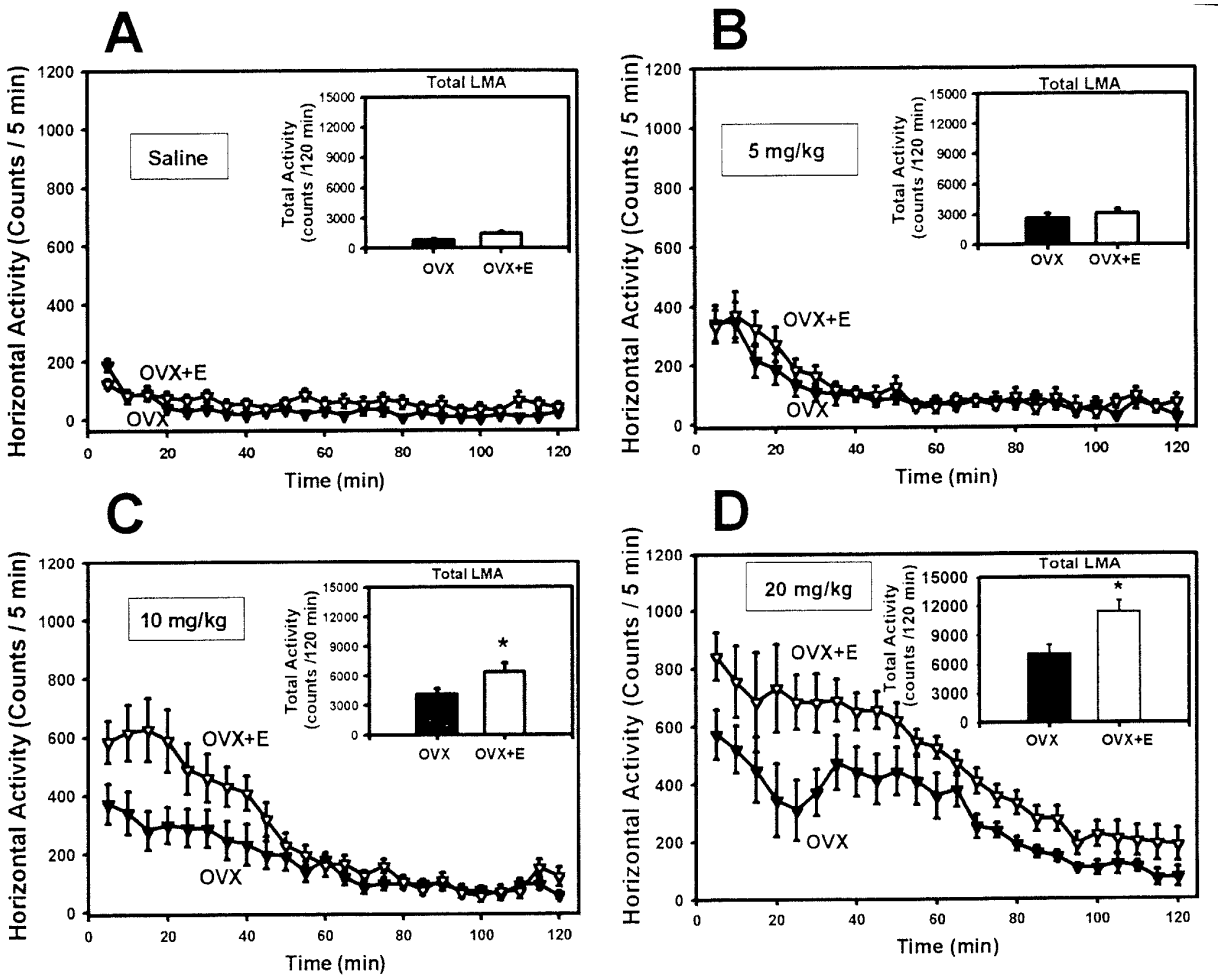

Figure 7. Effects of 17β-estradiol on cocaine-evoked horizontal locomotor activity (LMA) in ovariectomized (OVX) rats. The time course of mean ($\pm SEM$) horizontal activity counts/5 min for the 120-min session is shown for OVX (closed bars) and OVX + estrogen (E) rats (open bars; n = 8/group, 8 groups) after administration of saline (A) or 5 mg/kg (B), 10 mg/kg (C), or 20 mg/kg (D) cocaine. Mean ($\pm SEM$) total activity counts for the entire 120-min session are shown for each group as an insert bar plot (* $p < .05$ vs. OVX).

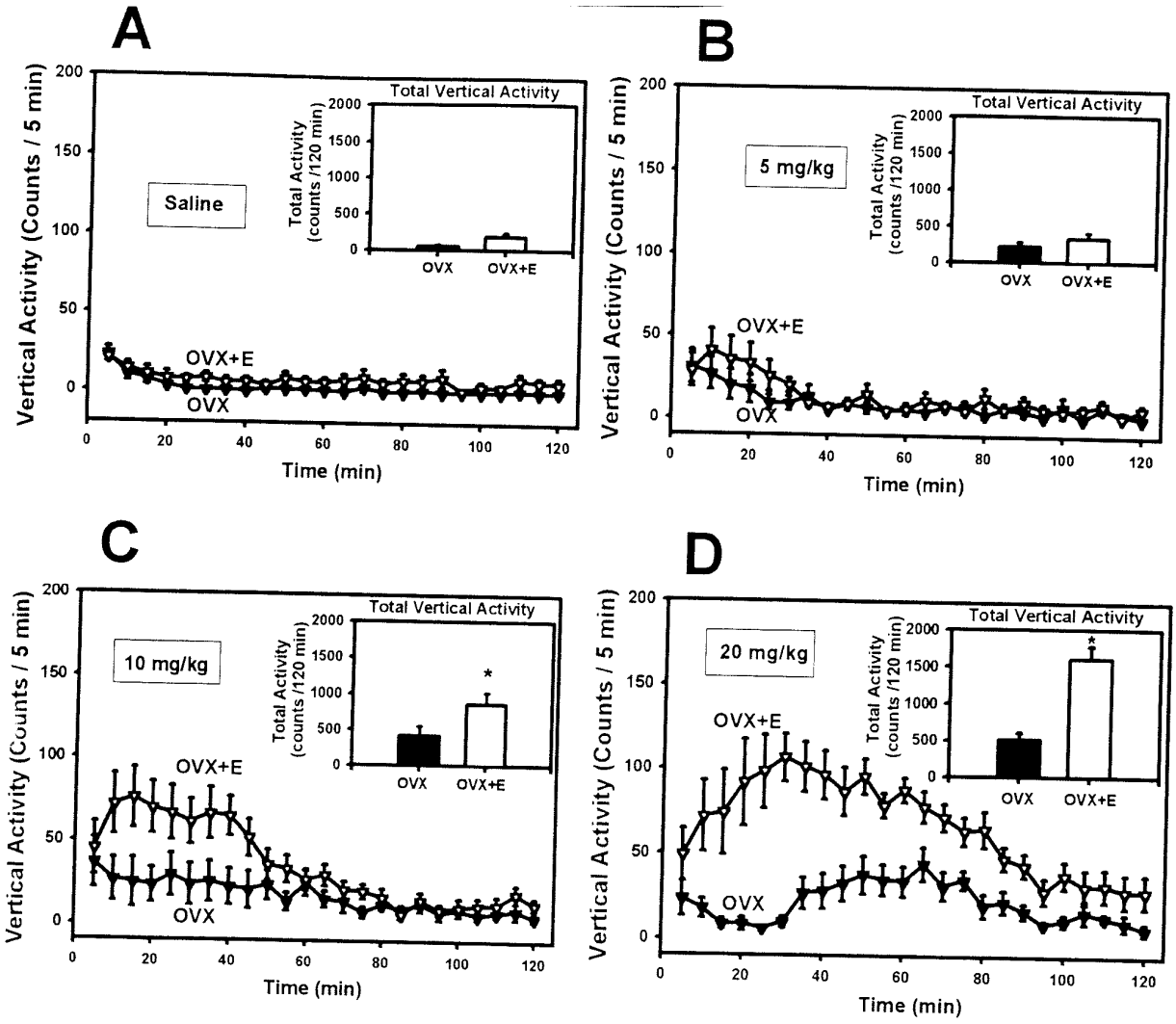

Figure 8. Effects of 17β-estradiol on cocaine-evoked vertical locomotor activity of ovariectomized (OVX) rats. The time course of mean ($\pm SEM$) vertical activity counts/5 min for the 120-min session is shown for OVX (closed bars) and OVX + estrogen (E) rats (open bars; n = 8/group, 8 groups) after administration of saline (A) or 5 mg/kg (B), 10 mg/kg (C), or 20 mg/kg (D) cocaine administration. Mean ($\pm SEM$) total activity counts for the entire 120-min session are shown for each group as an insert bar plot ($^* p < .05$ vs. OVX).

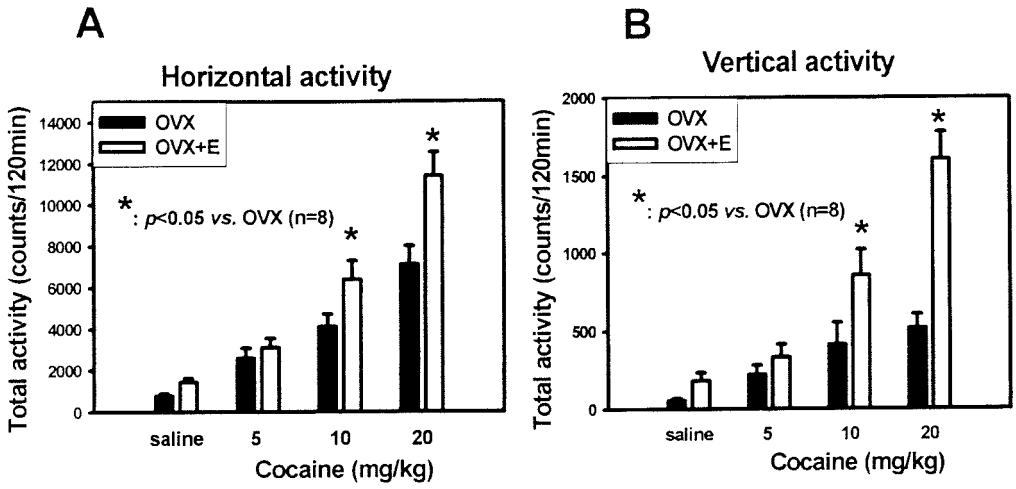

Figure 9. Summary data for 17β-estradiol effects on the dose–response for locomotor activity induced by cocaine in ovariectomized (OVX) rats. Mean (±*SEM*) total horizontal (A) and vertical (B) locomotor activity for the 120-min session are shown for OVX (closed bars) and OVX + estrogen (E) rats (open bars; n = 8/group, 16 groups) after administration of saline or 5, 10, or 20 mg/kg cocaine (* $p < .05$ vs. OVX).

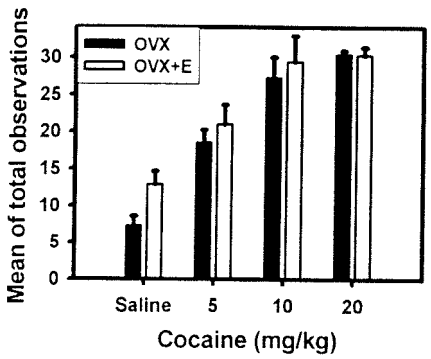

Figure 10. Effects of 17β-estradiol on observed behaviors induced by cocaine in ovariectomized (OVX) rats. Total (±*SEM*) scores of observations for the 120-min session are shown for OVX (closed bars) and OVX + estrogen (E) rats (open bars; n = 8/group, 8 groups) after administration of saline or 5, 10, or 20 mg/kg cocaine.