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Amphetamine Treatment During the Preweanling Period Produces Enduring Changes in Striatal Protein Kinase A Activity

CYNTHIA A. CRAWFORD,* ARTURO R. ZAVALA,* PATRICK E. KARPER,* ROBERT L. COLLINS,*[1] TORRAN LORING-MEIER,*[2] JOSEPH B. WATSON† AND SANDERS A. MCDOUGALL*

*Department of Psychology, California State University, San Bernardino, CA 92407; †Mental Retardation Research Center, University of California–Los Angeles, 760 Westwood Plaza, Los Angeles, CA 90024

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CRAWFORD, C. A., A. R. ZAVALA, P. E. KARPER, R. L. COLLINS, T. E. LORING-MEIER, J. B. WATSON AND S. A. MCDOUGALL. *Amphetamine treatment during the preweanling period produces enduring changes in striatal protein kinase A activity.* PHARMACOL BIOCHEM BEHAV **66**(4) 835–840, 2000.—The purpose of this study was to determine whether chronic exposure to amphetamine during the preweanling period causes enduring changes in behavioral and neuronal functioning. In two experiments rats were injected with saline or amphetamine (2.5 or 5.0 mg/kg) on postnatal days (PD) 11–15. Rats then received a challenge injection of saline or 2.5 mg/kg amphetamine on PD 23 or PD 90 and locomotor activity was measured. After behavioral assessment, rats were killed, and their dorsal striata and nucleus accumbens were dissected and later assayed for protein kinase A (PKA) activity. Interestingly, amphetamine treatment during the preweanling period produced an enduring decline in dorsal striatal and accumbal PKA activity that was still apparent in adulthood. These reductions in PKA activity were not related to the occurrence of locomotor sensitization, because rats did not exhibit a sensitized locomotor response when challenged with amphetamine at PD 23 or PD 90. © 2000 Elsevier Science Inc.

Protein Kinase A Amphetamine Behavioral sensitization Ontogeny

IN adult rats, chronic treatment with psychostimulants alters neuronal functioning in a variety of ways. For example, repeated treatment with amphetamine or cocaine produces long-term changes in cyclic adenosine monophosphate (cAMP) signal transduction systems (1,4,9,30,39). These psychostimulant-induced changes in cAMP functioning include modifications in G-protein sensitivity (4,10,33,36,37), subunit levels (27,38), adenylyl cyclase activity (1,9,30), and protein kinase A (PKA) activity (8,9,39). The mechanisms responsible for these alterations in cAMP functioning have not been determined, but it is possible that they are due to changes in dopamine D_1-like receptor sensitivity. Evidence supporting this idea is threefold: first, D_1-like receptors play an important role in mediating the neural effects of psychostimulants (6); second, acute D_1-like receptor stimulation increases adenylyl cyclase activity (18,34); and, third, repeated amphetamine treatment decreases D_1-stimulated adenylyl cyclase activity (1,30). Thus, repeated psychostimulant administration may lead to a desensitization of D_1-like receptors and, ultimately, a decrease in DA-stimulated adenylyl cyclase activity. Such changes in adenylyl cyclase activity would presumably affect other important components of the cAMP signal transduction system, including cAMP levels and PKA activity.

Besides affecting these various neuronal processes, repeated psychostimulant treatment produces a phenomenon called behavioral sensitization, which is manifested as an augmented behavioral response to a stimulant drug (17,29). Both young and adult rats exhibit behavioral sensitization

Requests for reprints should be addressed to Dr. C. A. Crawford, California State University, Department of Psychology, San Bernardino, CA 92407.
[1]Current address: Department of Psychology, University of Houston, 4800 Calhoun Road, Houston, TX 77204.
[2]Current address: Department of Psychology, Loma Linda University, 11130 Anderson Street, Loma Linda, CA 92350.

(17,21,22,29,40,43), although the sensitization exhibited by younger animals may differ from adults in some important respects (13,20,22,41). For example, there is contradictory evidence concerning the persistence of behavioral sensitization in young rats, with some researchers reporting that a sensitized locomotor response will persist for at most a few days after final drug exposure (13,20,22,41), whereas other researchers have found that sensitization will persist for substantially longer periods (35,44). Although many explanations for behavioral sensitization have been proposed, there is accumulating evidence indicating that stimulant-induced alterations in cAMP mechanisms are necessary for behavioral sensitization (1,9,24,39). It has not been determined, however, whether ontogenetic changes in the functioning of cAMP signal transduction systems are responsible for age-dependent differences in behavioral sensitization.

The purpose of the present study was to determine whether exposure to amphetamine during the preweanling period produces enduring changes in behavioral and neuronal functioning that persist across an extended drug abstinence period. More specifically, rats were repeatedly administered amphetamine or saline during the preweanling period and then challenged with amphetamine or saline at either postnatal day (PD) 23 or PD 90. On these test days both locomotor activity and PKA activity were assessed. Locomotor activity was measured to assess the presence of a sensitized behavioral response. PKA activity was measured because it is a reliable index of intracellular cAMP functioning (14,42), and is known to be altered by repeated psychostimulant treatment (26).

METHOD

Animals

A total of 112 rats of Sprague–Dawley descent (Harlan, Indianapolis, IN) were born in the vivarium at California State University, San Bernardino. Litters were culled to 10 pups at 3 days of age. Rats tested at PD 23 were housed with their littermates and dam until testing, whereas rats tested at PD 90 were weaned at PD 25 and housed with same-sexed littermates. Care was taken to ensure that an equal number of male and female rats were placed in each experimental condition. Food and water were available ad lib. The colony room was maintained at 22−24°C, and kept under a 12 L:12 D cycle. Subjects were treated according to the National Institute of Health guidelines for the care and use of laboratory animals ("Principles of Laboratory Animal Care," NIH Publication #85-23).

Drugs

S(+)-amphetamine sulfate (Research Biochemicals, Natick, MA) was dissolved in saline and injected intraperitoneally (IP) at a volume of 5 ml/kg for preweanling rats and at 1 ml/kg for adult rats.

Procedure

Experiment 1. Starting at PD 11, 32 male rats and 32 female rats were pretreated with saline or amphetamine (2.5 mg/kg, IP) in their home cage for 5 consecutive days. After 7 or 74 abstinence days (i.e., on PD 23 or PD 90), rats were given a challenge injection of saline or amphetamine (2.5 mg/kg, IP). Immediately after being injected, rats were placed in white plywood activity chambers (30 × 30 × 42 cm) with lines dividing the floors into four equal quadrants. After 5 min, line crosses (a measure of horizontal locomotor activity) were measured continuously across a 30-min testing session. Rats were killed 30 min after testing and their dorsal striata (i.e., caudate-putamen) were dissected and frozen at −80°C until time of assay. In summary, four groups of rats ($n = 16$) were pretreated and challenged with the following sequence of drugs (pretreatment–challenge): saline–saline, amphetamine–saline, saline–amphetamine, and amphetamine–amphetamine. These four groups were further subdivided with half of the rats receiving their challenge injection at PD 23 and the other half at PD 90 ($n = 8$ per group).

Experiment 2. Starting at PD 11, 24 male rats and 24 female rats were pretreated with saline or amphetamine (2.5 or 5.0 mg/kg, IP) in their home cage for 5 consecutive days. After 7 or 74 abstinence days (i.e., on PD 23 or PD 90), rats were given a challenge injection of amphetamine (2.5 mg/kg, IP). Immediately after being injected, rats were placed in white plywood activity chambers proportioned according to body length (PD 23: 17 × 17 × 24 cm; PD 90: 30 × 30 × 42 cm). After 5 min, line crosses were measured continuously across a 120-min testing session. Rats were killed immediately after testing, and their dorsal striata and nucleus accumbens were dissected and frozen at −80°C until time of assay. In summary, three groups of rats ($n = 16$) were pretreated with saline, 2.5 mg/kg amphetamine, or 5.0 mg/kg amphetamine. These three groups were further subdivided with half of the rats receiving their challenge injection at PD 23 and the other half at PD 90 ($n = 8$ per group).

PKA Assay

Frozen tissue sections were placed in homogenization buffer [50 mM HEPES (pH 7.4), 10 mM $MgCl_2$, 10 mM benzamide, 100 ng/ml leupeptin, 100 ng/ml aprotinin, 1 mM EDTA, and 1 mM EGTA] and homogenized using a handheld homogenizer. Protein concentrations were determined using the Bio-Rad Protein assay (Bio-Rad Laboratories, Hercules, CA), based on the method of Bradford (3), using bovine serum albumin as a standard. Duplicate tissue homogenates containing 4 μg of protein for each subject were incubated for 3 min at 30°C in phosphorylation buffer [50 mM Tris (pH 7.4), 10 mM $MgCl_2$, 1 mM EDTA, 1 mM EGTA, and 10 mM DTT] containing 15 μg of histone and 50 μM [γ-^{32}P]ATP (ICN, Costa Mesa, CA). The buffer also contained either 8-bromo-cyclic AMP (5 μM) or PKI (5 μg/reaction). Following incubation, the phosphorylation mixture was blotted on phosphocellulose filter paper. The filter paper was washed for 90 min, air dried, and then placed in scintillation fluid and quantified by liquid scintillation spectrophotometry. cAMP-dependent PKA activity was defined as the difference between PKA activity in the presence of 8-bromo-cAMP and that measured in the presence of PKI.

Statistical Analyses

To assess drug-induced behavioral effects, line cross data from Experiment 1 were analyzed using two 2 × 2 (pretreatment drug × challenge drug) ANOVAs, whereas line cross data from Experiment 2 were analyzed using two 3 × 8 (pretreatment drug × 15-min time blocks) ANOVAs. Separate ANOVAs were done for each age group. In each of these analyses litter effects were controlled by using within-litter statistical procedures (i.e., a within analysis using one value/condition/litter) (45). To assess sex-induced behavioral effects, line cross data from both experiments were reanalyzed

with sex being included as a factor in the statistical analyses. In these analyses, litter effects were not controlled through statistical procedures because there was not a sufficient number of subjects per litter to provide one male and female rat for each drug and age group. In all cases, however, only one subject per litter was placed into a particular group.

For both statistical and presentation purposes PKA data were transformed to percent of saline controls [see (16,28, 39)]. PKA data from Experiment 1 were analyzed using two 2 × 2 (pretreatment drug × challenge drug) randomized block ANOVAs, whereas PKA data from Experiment 2 were analyzed using two one-way (pretreatment drug) randomized block ANOVAs. A total of four to six assays were done for each experiment, with each assay being treated as a separate block. This procedure allowed for the variance between assays to be removed from the analysis (19). Each PKA assay included tissue samples from only one litter; thus, litter was treated as a random factor in these statistical analyses. Additional statistical analyses showed that PKA activity did not vary according to sex, so these data are not presented in the Results section. Post hoc analysis of line cross and PKA data was made using Dunnett and Tukey tests ($p < 0.05$).

RESULTS

Experiment 1

Rats challenged with 2.5 mg/kg amphetamine on PD 23 or PD 90 had more line crosses than rats challenged with saline (see Fig. 1), [challenge drug main effect, PD 23, $F(1, 7) = 244.76$, $p < 0.001$; PD 90, $F(1, 7) = 115.91$, $p < 0.001$]. Amphetamine (2.5 mg/kg) pretreatment on PD 11−15 did not affect the locomotor activity of rats tested on PD 23 or PD 90. Separate statistical analyses showed that female rats had more line crosses than male rats when the test day occurred at PD 90 (see Table 1) [sex main effect, $F(1, 24) = 5.37$, $p < 0.05$]. There were no sex differences when rats were tested at PD 23 (see Table 1).

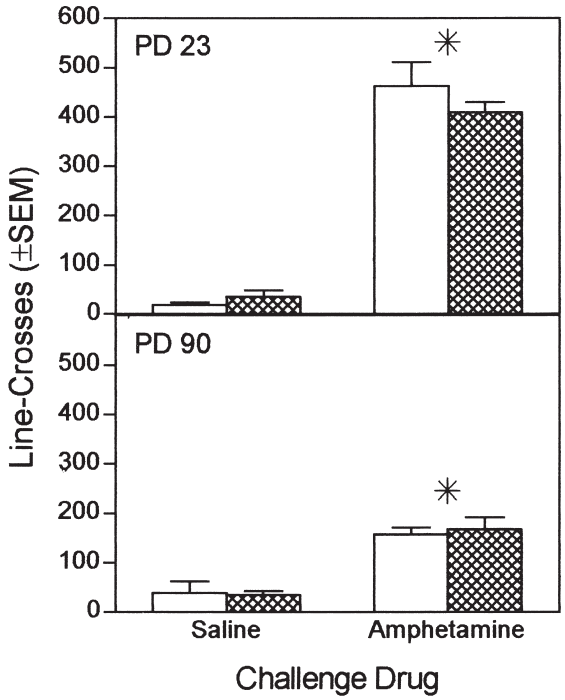

FIG. 1. Mean line crosses of rats given a challenge injection of saline or amphetamine (2.5 mg/kg, IP) on the test day. Rats had been pretreated with saline (open bars) or 2.5 mg/kg amphetamine (cross-hatched bars) for 5 consecutive days starting on PD 11. Testing sessions lasted 30 min and occurred on PD 23 or PD 90. *Significantly different from rats given a challenge injection of saline on the test day ($p < 0.05$).

TABLE 1

MEAN LINE CROSSES (±SEM) OF MALE AND FEMALE RATS GIVEN A CHALLENGE INJECTION OF SALINE OR AMPHETAMINE (2.5 mg/kg, IP) ON THE TEST DAY

	Sex	
Challenge Drug	Males	Females
PD 23		
Saline	28.25 (±20.8)	27.25 (±10.2)
Amphetamine	413.50 (±11.3)	458.38 (±8.5)
PD 90		
Saline	32.12 (±7.4)	42.12 (±22.5)*
Amphetamine	127.75 (±17.2)	195.25 (±13.8)*

Testing sessions lasted 30 min and occurred on PD 23 or PD 90. These are the same rats as shown in Fig. 1.

*Significantly different from male rats ($p < 0.05$).

Amphetamine (2.5 mg/kg) pretreatment on PD 11−15 significantly reduced the dorsal striatal PKA activity of rats tested on PD 23 or PD 90 (see Table 2), [pretreatment drug main effect, PD 23, $F(1, 9) = 6.66$, $p < 0.05$; PD 90, $F(1, 9) = 9.51$, $p < 0.05$]. Amphetamine-induced reductions in PKA activity were apparent in rats given a test day injection of either saline or amphetamine.

Experiment 2

Once again, amphetamine (2.5 or 5.0 mg/kg) exposure on PD 11−15 did not affect the locomotor activity of rats tested on PD 23 or PD 90 (see Fig. 2). Line crosses of both age groups increased across the first 45 min of testing and then declined over the remainder of the testing session, [time main effect, PD 23, $F(7, 49) = 30.83$, $p < 0.001$; PD 90, $F(7, 49) = 45.52$, $p < 0.001$]. When tested at PD 90, female rats exhibited

TABLE 2

DORSAL STRIATAL PKA ACTIVITY (±SEM) OF RATS KILLED 30 MIN AFTER TESTING ON PD 23 OR PD 90

	PKA Activity	
Drug Administration (Pretreatment–Challenge)	PD 23	PD 90
Saline–Saline	100.0 (±39.2)	100.0 (±8.1)
Amphetamine–Saline	80.3 (±39.9)*	67.3 (±8.5)*
Saline–Amphetamine	101.6 (±54.0)	98.8 (±14.1)
Amphetamine–Amphetamine	66.7 (±31.7)*	80.5 (±9.8)*

Rats were pretreated with saline or amphetamine (2.5 mg/kg, IP) for 5 consecutive days starting on PD 11, and given a challenge injection of saline or amphetamine (2.5 mg/kg, IP) on the test day. PKA activity (nmol/min/mg protein) data are expressed as percent of saline–saline controls.

*Significantly different from saline-pretreated rats ($p < 0.05$).

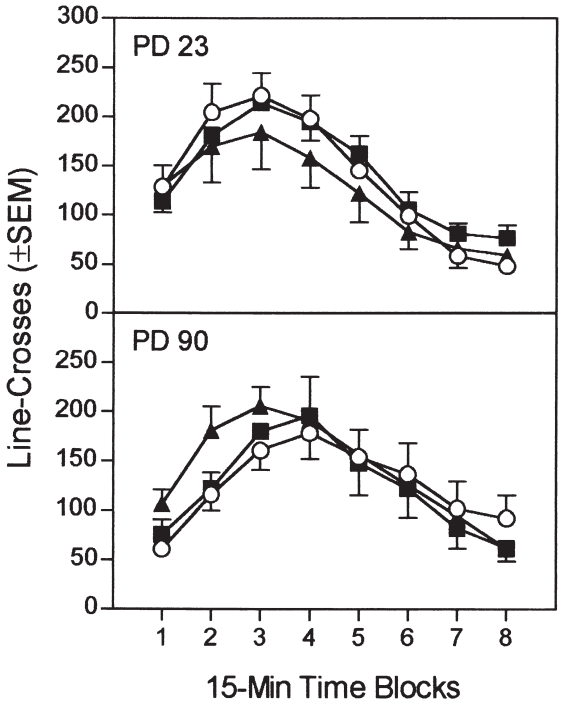

FIG. 2. Mean line crosses of rats given a challenge injection of amphetamine (2.5 mg/kg, IP) on the test day. Rats had been pretreated with saline (open circles), 2.5 mg/kg amphetamine (filled triangles), or 5.0 mg/kg amphetamine (filled squares) for 5 consecutive days starting on PD 11. Testing sessions lasted 120 min and occurred on PD 23 or PD 90.

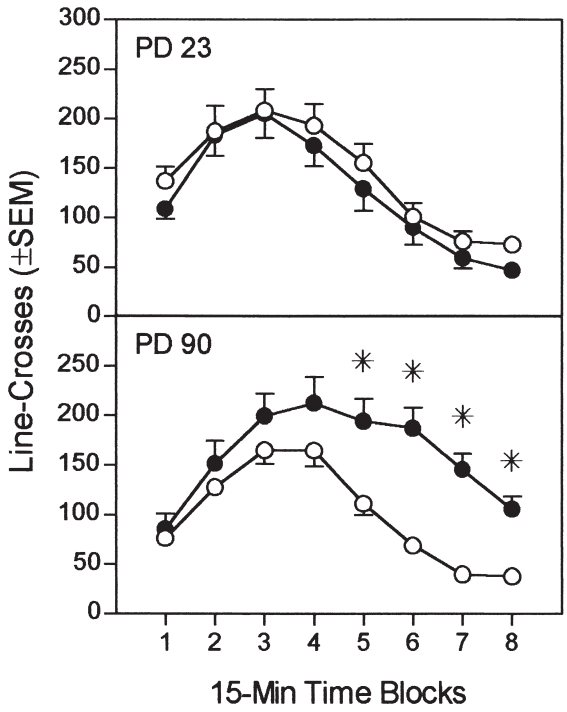

FIG. 3. Mean line crosses of male (open circles) and female (filled circles) rats given a challenge injection of amphetamine (2.5 mg/kg, IP) on the test day. Testing sessions lasted 120 min and occurred on PD 23 or PD 90. These are the same rats as shown in Fig. 2. *Significantly different from male rats ($p < 0.05$).

more line crosses than male rats (see Fig. 3) [sex main effect, $F(1, 18) = 11.76$, $p < 0.01$; sex × time interaction, $F(7, 126) = 6.35$, $p < 0.001$]. Line crosses of rats tested at PD 23 did not vary due to sex (see Fig. 3).

When assayed on PD 90, dorsal striatal PKA activity was depressed in rats given 2.5 or 5.0 mg/kg amphetamine on PD 11–15 (see Table 3)[pretreatment drug main effect, $F(2, 10) = 5.75$, $p < 0.05$]. Similarly, amphetamine (2.5 or 5.0 mg/kg) exposure on PD 11–15 significantly reduced the accumbal PKA activity of rats tested on PD 90 (see Table 3) [pretreatment drug main effect, $F(2, 10) = 7.18$, $p < 0.05$]. Early exposure to 2.5 mg/kg amphetamine, but not 5.0 mg/kg amphetamine, decreased the dorsal striatal PKA activity of rats tested on PD 23 [pretreatment drug main effect, $F(2, 10) = 9.69$, $p < 0.01$]. It is uncertain why only the lower dose of amphetamine affected dorsal striatal PKA activity in these younger animals. On the same test day (i.e., PD 23), PKA activity in the nucleus accumbens was not affected by amphetamine pretreatment.

DISCUSSION

The purpose of the present study was to determine whether repeated exposure to amphetamine during the preweanling period would produce long-term alterations in PKA activity and locomotor activity (i.e., induce behavioral sensitization). Rats given amphetamine on PD 11–15 had reduced levels of dorsal striatal PKA activity when measured at PD 23 and PD 90. Early amphetamine exposure (2.5 or 5.0 mg/kg) also reduced PKA activity in the nucleus accumbens at PD 90, but not at PD 23. The reason for this ontogenetic difference is uncertain, although the overproduction of accumbal D_1-like receptors at this age (25) may be involved. That early amphetamine exposure produced long-term reductions in PKA activity is interesting, and could be caused by a persistent desensitization or downregulation of D_1-like recep-

TABLE 3

DORSAL STRIATAL AND ACCUMBAL PKA ACTIVITY (±SEM) OF RATS KILLED IMMEDIATELY AFTER TESTING ON PD 23 OR PD 90

Drug Administration (Pretreatment)	PKA Activity	
	PD 23	PK 90
Dorsal striatum		
Saline	100.0 (±20.8)	100.0 (±10.2)
Amphetamine (2.5 mg/kg)	59.3 (±11.3)*	76.2 (±8.5)*
Amphetamine (5.0 mg/kg)	89.0 (±21.6)	80.6 (±9.1)*
Nucleus accumbens		
Saline	100.0 (±24.1)	100.0 (±18.3)
Amphetamine (2.5 mg/kg)	92.6 (±20.0)	77.2 (±15.3)*
Amphetamine (5.0 mg/kg)	111.9 (±27.6)	70.3 (±10.0)*

Rats were pretreated with saline or amphetamine (2.5 or 5.0 mg/kg, IP) for 5 consecutive days starting on PD 11, and given a challenge injection of amphetamine (2.5 mg/kg, IP) on the test day. PKA activity (nmol/min/mg protein) data are expressed as percent of saline controls.

*Significantly different from saline-pretreated rats ($p < 0.05$).

tors. The cAMP-dependent PKA pathway is positively coupled to D_1-like receptors (18,34), so desensitization of these receptors could decrease PKA activity. Consistent with this idea, nondevelopmental studies have shown that repeated amphetamine treatment depresses striatal D_1-stimulated adenylyl cyclase activity (1,30). Alternatively, amphetamine-induced reductions in PKA activity could be caused by a persistent sensitization or upregulation of D_2-like receptors. The cAMP-dependent PKA pathway is negatively coupled to D_2-like receptors (18,34), so sensitization of these receptors would also decrease PKA activity. Evidence consistent with this explanation is available, as a haloperidol-induced upregulation of D_2-like receptors decreases both basal and DA-stimulated adenylyl cyclase activity (32). Presumably, these reductions in adenylyl cyclase activity would depress both cAMP levels and PKA activity. Regardless of the explanation, the present results show that amphetamine exposure during the preweanling period produces a long-term decrease in striatal and accumbal PKA activity.

The behavioral relevance of this amphetamine-induced decline in PKA activity is uncertain, because rats did not show a sensitized behavioral response when challenged with amphetamine after either 7 or 74 drug abstinence days (i.e., on PD 23 or PD 90). Of course, it is possible that the decline in PKA activity may have been responsible for the lack of behavioral sensitization at PD 23 and PD 90, but this seems unlikely, because stimulant-induced reductions in striatal adenylyl cyclase are correlated with robust stereotyped sensitization in adult rats (1). Interestingly, Nestler and colleagues (39) have shown that adult rats treated chronically with cocaine exhibit enhanced PKA activity in the nucleus accumbens, but not in the dorsal striatum. Thus, stimulant-induced increases, but not decreases, in accumbal PKA activity may be important for the expression of locomotor sensitization (24,39).

In the behavioral sensitization literature there is conflicting evidence about whether young rats will exhibit a sensitized locomotor response after more than a few drug abstinence days (13,20,22,35,41,44). When these studies are considered together, it appears that young rats are capable of showing behavioral sensitization after an extended abstinence period (7 days or more) if given a substantial number of drug pretreatments in a novel test chamber (35,44). Persistence of the sensitized response is typically not observed when either few drug administrations are given (22) or when the drug is not administered in a novel test environment (13,20,35,41,44). It is possible that either, or both, of these factors were responsible for the lack of behavioral sensitization in the present study.

Although dorsal striatal and accumbal PKA activity did not vary according to sex, there was evidence of age-dependent sex differences when locomotor activity was examined. Specifically, female rats exhibited more locomotor activity than male rats when testing occurred at PD 90, but not at PD 23. This pattern of results has been reported many times before, as adult female rats typically show more locomotion and rotational behavior than adult males (5,12,31). Young rats, in contrast, do not exhibit sex-related differences in locomotor activity until between 6–8 weeks of age (7,11,15).

In summary, the results of this study show that chronic amphetamine treatment during the preweanling period causes enduring changes in dorsal striatal and accumbal PKA activity. It does not appear that these alterations in PKA activity are necessary for the occurrence of locomotor sensitization; however, it is unknown whether amphetamine-induced changes in PKA activity impact reward-related learning, addiction, or memory processes [see (2,23,26)].

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