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Protracted 'Pro-Addictive' Phenotype Produced in Mice by Pre-Adolescent Phenylpropanolamine

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For decades, the sympathomimetic phenylpropanolamine (PPA; ±-norepinephrine) was an active ingredient found in popular children's over-the-counter (OTC) cold, cough, and allergy medications. To examine the possibility that pre-adolescent PPA exposure may induce neuroadaptations that influence behavioral and neurochemical responding to cocaine, C57BL/6J mice were pretreated with PPA (0–40 mg/kg) during postnatal days 21–31. The behavioral and neurochemical responses to acute and repeated cocaine (4 × 15 mg/kg) were then assessed in adulthood when the mice were 10 weeks of age. Whereas pre-adolescent PPA exposure did not influence the acute locomotor response to 15 mg/kg cocaine, PPA pre-exposure dose-dependently enhanced the expression of cocaine-induced place conditioning, reduced the expression of locomotor sensitization, but did not influence cocaine-induced stereotypy. Pre-adolescent PPA exposure completely prevented the capacity of cocaine to elevate extracellular levels of catecholamines in the nucleus accumbens, but facilitated the development of cocaine-induced glutamate sensitization. Neither acute nor repeated cocaine altered extracellular GABA levels in the accumbens of control mice; however, 15 mg/kg cocaine lowered GABA levels by approximately 40% in PPA pretreated mice and this effect showed tolerance with repeated cocaine administration. These data provide the first evidence that early exposure to an OTC compound produces protracted effects upon cocaine-induced changes in nucleus accumbens neurotransmission that may contribute to a 'pro-addictive' phenotype in adulthood.
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INTRODUCTION

The marked increase in the prescription of psychotropic drugs to children (Zito *et al*, 2000) has sparked a considerable amount of research concerning the enduring psychobiological effects of pre-adolescent (juvenile) prescription drug exposure, particularly as they relate to the behavioral sensitivity to illicit drugs in adulthood (cf., Carlezon and Konradi, 2004). However, no study to date has examined the possibility that juvenile exposure to a psychotropic over-the-counter (OTC) medication may alter the function of brain reward circuits and influence subsequent susceptibility to addiction-related behaviors. Phenylpropanolamine (PPA; ±-norephedrine) is a non-selective adrenergic receptor agonist and norepinephrine reuptake inhibitor (Hoffman, 2001) that was widely available for decades in popular children's OTC cold, cough, and allergy medications (eg, Alka-Seltzer® Plus Children's Cold Elixer and Orange, Cherry, Berry, and Yellow Triaminic® 3D preparations). Moreover, PPA is a major metabolite of methylphenidate (Patrick *et al*, 1981), the most highly prescribed psychomotor stimulant for the treatment of Attention-Deficit/Hyperactivity Disorder (eg, Zito *et al*, 2000). Despite its prevalence and sympathomimetic profile, the biopsychological effects of PPA have received very little experimental attention. Whereas PPA does not elicit locomotor hyperactivity in rats or maintain self-administration behavior in monkeys trained to self-administered cocaine (Eisenberg *et al*, 1987; Mittleman *et al*, 1993; Woolverton *et al*, 1986), it substitutes for amphetamine in rat self-administration studies (Wellman *et al*, 1989). Moreover, PPA substitutes for illicit psychomotor stimulants in drug discrimination studies employing both human and nonhuman primates, as well as rodents (Chait *et al*, 1988; Lee *et al*, 1989; Mariathasan and Stolerman, 1992; Woolverton *et al*, 1986).

As PPA and illicit stimulant drugs share subjective and reinforcing properties, we assessed the protracted effects of pre-adolescent exposure to PPA upon the behavioral and neurochemical responses to cocaine in adulthood. We found that the administration of PPA to juvenile mice elicits enduring alterations in nucleus accumbens (NAC) neurotransmission that may culminate in a 'pro-addictive'

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phenotype in later life, posing OTC sympathomimetic drug exposure as a potential early environmental factor that may contribute to addiction vulnerability.

MATERIALS AND METHODS

Subjects

C57BL/6J male and female mice were obtained from Jackson Laboratory (Bar Harbor, ME) and used to generate male and female pups for the present experiments. For each experiment, pups from three different litters were weaned on postnatal day (PND) 21 and housed in same-sex pairs in brown, clear standard polyethylene cages (15 cm wide × 23 cm long × 16 cm high) with woodchip bedding, under a regular 12-h light cycle (lights on 0700 hours). Between PND 21 and 30, a time corresponding to pre-adolescence in rodents (Adriani *et al*, 2004; Cirulli and Laviola, 2000; Spear and Brake, 1983), pups were administered, once daily, intraperitoneal (i.p.) injection of either saline vehicle (vol = 0.01 ml/g) or 5, 10, and 40 mg/kg PPA. These doses of PPA are in the range of those used previously to examine the behavioral or neurochemical effects of PPA in adult rodents (Eisenberg *et al*, 1987; Mariathasan and Stolerman, 1992; Mittleman *et al*, 1993; Rushing *et al*, 1993; McMahon and Wellman, 1996). As PPA is an anorectic (eg, Eisenberg *et al*, 1987; Mittleman *et al*, 1993; Wellman, 1990), body weight was recorded daily before each injection. All injections, behavioral and neurochemical testing occurred during the light phase of the circadian cycle, between 0900 and 1600 h. Statistical analysis of the behavioral and neurochemical data revealed no main effects of, or interactions with, the sex factor. All experimental protocols were consistent with the guidelines of the NIH Guide for Care and Use of Laboratory Animals (NIH Publication No. 80-23, revised 1996) and approved by the Institutional Animal Care and Use Committee of the University of California, Santa Barbara, CA.

PPA-Induced Changes in Locomotor Activity during PND 21–30

To determine whether or not PPA induced a psychoactive effect in pups, the locomotor response to PPA (0, 5, 10, and 40 mg/kg) was measured on injections 1, 5, and 10 of repeated treatment (PNDs 21, 25, and 30, respectively). Locomotor activity was monitored in a noncolony room containing eight Plexiglas activity chambers (22 × 43 × 33 cm) (Omnitech Electronics, Columbus, OH). A series of 16 photobeams (eight on each horizontal axis) tabulated horizontal movements and estimated the total distance traveled by the pups (in cm). These chambers were interfaced to a Digiscan monitor (Omnitech Electronics, Columbus, OH) and an IBM PC computer provided automated recording of motor behavior. On injections 1, 5, and 10 of repeated PPA administration, pups were weighed, injected i.p. with the appropriate pretreatment dose and placed immediately into the activity chambers for 2 h. On the intervening days, mice were weighed, injected, and returned to their home cages. Following the last PPA injection, mice were left undisturbed in the colony room until they were 10 weeks of age. Sample sizes ranged from 16 to 18/dose.

Place Conditioning and Locomotor Activity

At 10 weeks of age, the capacity of four i.p. injections of 15 mg/kg cocaine (Sigma-Aldrich, St Louis, MO) to induce place-conditioning and locomotor sensitization was assessed using a biased place conditioning procedure identical to that described previously for mice (Szumlinski *et al*, 2002, 2004a, 2005b). As also discussed previously (eg, Szumlinski *et al*, 2002), a biased place-conditioning procedure was used to reduce the probability of observing a ceiling effect upon approach behavior (Shimosato and Ohkuma, 2000). The apparatus employed in the present study enabled the assessment of three aspects of behavior: motor activity, anxiety, and cocaine-induced place conditioning. The apparatus was constructed of Plexiglas (46 cm long × 24 cm high × 22 cm wide) and consisted of two chambers that were divided into two distinct compartments by a removable divider. One compartment was painted black, contained corncob bedding and was covered by an opaque black lid; the other compartment was painted white, contained no bedding and was covered by a translucent white lid. The chambers were equipped with two photocells each, which were aligned with a 0.5-inch unpainted strip that ran along each wall of the apparatus to allow detection of the animals. The photocells were interfaced with a digital analyzer (Med Associates Inc., St Albans, VT) and a PC-type computer which provided information automatically as to the time spent in each compartment of the chamber and the number of photocell beam breaks, the latter of which was used to index both spontaneous and cocaine-induced changes in locomotor activity (Lominac *et al*, 2005, 2006; Szumlinski *et al*, 2002, 2004a, 2005a–c).

Place conditioning proceeded in the following four sequential phases: preconditioning test 1 (Pre-Test1), preconditioning test 2 (Pre-Test2), conditioning, and postconditioning test (Post-Test). For all phases, mice were placed in the chambers for a total of 15 min, once daily. Following Pre-Test1, in which animals had free access to both compartments of the place-conditioning chambers, the mice were then given a second Pre-Test. No injections were administered on either Pre-Test. As the place-conditioning apparatus is essentially a black–white shuttle box, the time spent in the white compartment during Pre-Test1 served to index basal anxiety (Belzung *et al*, 1987) and the total number of beam breaks during Pre-Test1 served to index spontaneous locomotion in response to a novel environment. The time spent in the nonpreferred compartment on the Pre-Test2 served as the baseline to assess the effects of conditioning.

Conditioning was conducted over an 8-day period. Cocaine-paired groups received i.p. injections, on alternate days, with either saline (0.01 ml/g body weight) or cocaine (a generous gift from NIDA, Bethesda, MD) and were placed in their preferred chamber on saline days or the nonpreferred chamber on cocaine days. To assist in the interpretation of shifts in the time spent in the nonpreferred chamber exhibited by cocaine-paired animals (eg, habituation to the aversiveness of the nonpreferred compartment;

Bardo and Bevins, 2000), control groups were included that received saline injections on each conditioning day. As per earlier cocaine studies (Szumlinski *et al*, 2002, 2004a), all cocaine/saline injections were administered in the experimental room and animals were placed in the place conditioning apparatus 5 min following injection. As described previously in other place-conditioning studies (Lominac *et al*, 2006; Szumlinski *et al*, 2002, 2004a, 2005a, c), locomotor activity was monitored during the first and last conditioning session when the mice were confined to the drug-paired compartment and the difference in drug-induced locomotor activity was used to index the development of cocaine-induced locomotor sensitization.

The day following the last conditioning session, a Post-Test was conducted. The Post-Test was identical to the Pre-Tests in that no injections were administered and mice had free access to both compartments. The difference in the time spent in the drug-paired compartment between the Post-Test and Pre-Test2 (occupancy difference) served to index the effects of cocaine/saline on place conditioning. Samples sizes ranged from 7 to 9 mice/PPA dose.

Cocaine-Induced Stereotypy

In addition to a sensitization of horizontal locomotion, the repeated administration of psychomotor stimulant drugs, such as cocaine, can induce a sensitization of intensely focused, stereotyped behaviors in rodents (including grooming, rearing, and sniffing) that can interfere with the expression of horizontal locomotion (eg, Kuczenski *et al*, 1991). Moreover, the expression of both stimulant-induced locomotion and stereotypy can become conditioned to the environment in which the drug is administered with repeated drug administration (Badiani *et al*, 2000; Badiani and Robinson, 2004; Browman *et al*, 1998; Pert *et al*, 1990; Robinson *et al*, 1998; Szumlinski *et al*, 1997). Thus, to determine whether or not PPA pretreatment altered the expression of cocaine-induced stereotypy and to assess the potential role for conditioned factors in mediating the observed effects of PPA pretreatment upon cocaine-induced locomotor sensitization, groups of mice were pretreated with either saline control or 40 mg/kg PPA during PNDs 21–30. At 10 weeks of age, mice were assigned to receive either three injections of saline or three injections of 15 mg/kg cocaine. As per the experiments above, these injections were administered every other day, but in this experiment, mice were returned immediately to their home cage following injection. On the fourth injection, mice were transported to a noncolony room, all injected with 15 mg/kg cocaine, and placed immediately into activity chambers (22 cm long × 24 cm high × 22 cm wide). The total distance traveled by the mice was recorded for 2 h using two digital video cameras, interfaced with a PC-type computer loaded with Any-MAZE software (Stoelting Company, Wood Dale, IL) and video recordings were later scored for the expression of stereotypy by an experimenter blind to the pretreatment of the animals. As conducted previously (Szumlinski *et al*, 2000b), stereotypy was scored for 30 s, in 10 min bins, using the following rating scale: 0 = asleep; 1 = awake but still; 2 = grooming or mild licking; 3 = continuous, exploratory locomotion along the horizontal plane for the entire 30 s without rearing; 4 = continuous, exploratory locomotion along the horizontal plane for the entire 30 s with rearing; 5 = bouts of locomotion along the horizontal plane/'darting'without rearing or sniffing; 6 = darting with bouts of rearing or sniffing; 7 = continuous sniffing for 30 s without horizontal locomotion or rearing; 8 = continuous sniffing for 30 s while rearing; 9 = patterned sniffing or head-bobbing in a fixed location for less than 30 s; 10 = patterned sniffing or head-bobbing in a fixed location for the entire 30 s; 11 = continuous gnawing or focused grooming; 12 = bizarre dyskinetic movements or seizures. Samples sizes = 7–8/group.

Intracranial Surgery and *In Vivo* Microdialysis

To relate the cocaine behavioral phenotype of the mice to cocaine-induced changes in neurotransmitters implicated in drug reward and psychomotor activation, *in vivo* microdialysis was conducted in the NAC of saline- and PPA-pretreated mice (PNDs 21–31), when the mice were 10 weeks of age. The 40 mg/kg PPA dose was selected for this experiment as it produced the largest effect upon cocaine-induced changes in behavior (Figure 2). At 9 weeks of age, mice were anesthetized under 4% isoflurane and were implanted bilaterally with a 20-gauge stainless-steel guide cannula (10 mm long) aimed above the NAC and the guide cannulae were fixed to the skull using dental resin as described previously (Lominac *et al*, 2005, 2006; Szumlinski *et al*, 2004a, 2005b, c). Following 1 week recovery, *in vivo* microdialysis was conducted. The procedures for probe construction and microdialysis were identical to those employed in previous studies of mice (Lominac *et al*, 2005, 2006; Szumlinski *et al*, 2004a, 2005b, c). Baseline dialysate was collected in 20-min fractions into 10 μl of a preservative solution for 1 h, and then animals were administered 15 mg/kg cocaine i.p. and dialysate collection continued for 3 h. Mice were returned to the colony room and received two more injections of 15 mg/kg cocaine, every other day, in a manner consistent with the injection regimen employed in the behavioral study. The morning of the fourth cocaine injection, the microdialysis procedures were repeated, with the exception that a new probe was inserted unilaterally on the opposite side of the head. Upon completion of the second session, mice were overdosed with pentobarbital, perfused with saline and then a 4% paraformaldehyde solution. Their brains were extracted, sliced along the coronal plane (50 μm thick) and stained with Cresyl violet for histological examination of probe placement. Only dialysate from mice with probe placements within the shell region of the NAC were analyzed for neurotransmitter content. Sample sizes ranged from 6 to 8/group.

HPLC Analysis of Monoamines and Amino Acids

The procedures and equipment for the high-pressure liquid chromatography (HPLC) analysis of dialysate samples were identical to those described recently (Lominac *et al*, 2006). The HPLC system consisted of a Coularray detector, a Model 542 autosampler and two Model 582 solvent delivery systems (ESA Inc., Bedford, MA), which enabled the sequential detection of monoamines and amino acids. For monoamines, the MD-TM mobile phase was employed (ESA Inc.), and neurotransmitters were separated using a

MD-150 × 3.2 column (15 cm; ESA Inc.). An ESA 5014B analytical cell with two electrodes was used for the electrochemical detection of monoamines—the reduction analytical electrode (E1, −150 mV), and an oxidation analytical electrode (E2, +220 mV). For amino acids, the mobile phase consisted of 100 mM NaH_2PO_4, 22% methanol (v/v), 3.5% acetonitrile (v/v), pH 6.75, and were separated using a CAPCELL PAK C18 mg column (5 cm; Shiseido Company Ltd, Tokyo, Japan). An ESA 5011A analytical cell with two electrodes was used for the electrochemical detection of amino acids, following precolumn derivatization with *o*-phthalaldehyde using the autosampler. The electrodes were set to +150 and +550 mV for oxidation. Neurotransmitter content in each sample was analyzed by peak height, and was compared with external standard curves (one for each neurotransmitter) for quantification using ESA Coularray for Windows software. Under our conditions, the monoamines norepinephrine, dopamine and serotonin eluted at 3.1, 5.2, and 11.5 min, respectively, whereas the amino acids glutamate and GABA eluted at 2.1 and 14.9 min, respectively.

Detection and Quantitation of Phenylpropanolamine in Plasma by LC/MS

To determine the pharmacokinetics of PPA in pre-adolescent mice, mice were injected with 40 mg/kg PPA on PND 21 and trunk blood was sampled at 15, 30, 90, and 180 min following drug injection. The samples were centrifuged and the plasma frozen at −80°C until shipped for liquid chromatography–mass spectrometry analysis by National Medical Services (NMS) Laboraties (Willow Grove, PA). An internal standard (D3-ephedrine) was added to 0.5 ml of sample followed by dilution with water and buffering to pH 6.0 with phosphate buffer. Samples were extracted using a solid-phase extraction column (Varian Certified®) and with 2% ammonium hydroxide in methanol as the final elution solvent. The elution solvent was evaporated to dryness and reconstituted with mobile phase. Samples were analyzed using a Micromass Platform II LC/MS with electrospray ionization, and a Hewlett Packard 1100 series HPLC with a Zorbax® SB-Phenyl 4.6 mm ID × 75 mm, 3.5 μm analytical column. A four-component gradient mobile phase was used starting with a high concentration of aqueous and transitioning to an organic (methanol)-rich mixture. Two ions were monitored for both analyte (152 and 137) and internal standard (151 and 169) to verify identification. Quantitations were performed using second-order, 1/*x* weighted, regression analysis of the response ratio of 152/151 *vs* concentration. Calibrators at concentrations of 5, 25, 50, 250, and 500 ng/ml ran along with Quality Control samples at target concentrations of 30 and 350 ng/ml. Mouse plasma samples were diluted with blank matrix so that the calculated concentrations were within the range of calibration. Phenylpropanolamine and D3-ephedrine eluted at approximately 4.1 and 5.0 min, respectively. Sample sizes ranged from 4 to 5 mice/time point.

Statistical Analyses

All data were analyzed by analyses of variance (ANOVA). As observed in an earlier study of behavioral and neurochemical effects of cocaine in mice (Szumlinski *et al*, 2000c, 2004a, 2005b), statistical analyses failed to detect any main effects of, or interactions with, the sex factor ($p > 0.05$). Thus, these data for male and female mice were collapsed for statistical analyses of PPA interactions with cocaine. Significant interactions were decomposed for simple effects, followed by LSD *post hoc* tests.

RESULTS

PPA Pharmacokinetics when Administered during Pre-Adolescence

To assess the pharmacokinetic profile of PPA in pre-adolescent mice, we injected PND 21 mice i.p. with the highest dose of PPA tested (40 mg/kg) and assessed for plasma levels at various times following injection. As depicted in Figure 1a, high levels of PPA were detected in plasma at 15 min post-injection and declined rapidly over the course of sampling in a manner independent of the sex of the animal (time effect, $F(3,26) = 11.21$; $p < 0.0001$; sex effect, $p = 0.33$; sex × time, $p = 0.84$).

PPA Inhibited Locomotor Activity during Pre-Adolescence

PPA administration (0–40 mg/kg, i.p.) inhibited the locomotor activity exhibited by pre-adolescent mice and this effect was independent of PPA dose (Figure 1b). The PPA-induced locomotor inhibition lessened with repeated administration, the level of locomotor activity exhibited by PPA mice remained below that of saline controls throughout treatment (PPA effect: $F(3,62) = 19.21$, $p < 0.0001$; PPA injection number effect: $F(2,124) = 34.36$, $p < 0.0001$; PPA × PPA injection number: $F(6,124) = 1.76$, $p = 0.11$).

PPA is an anorectic (Eisenberg *et al*, 1987; Mittleman *et al*, 1993; Wellman, 1990). Thus, we analyzed for the effects of PPA on weight gain in male and female pups during repeated treatment. As illustrated in Figure 1c and d, PPA treatment did not influence the gain in weight exhibited by either male or female pups during PNDs 21–30 in the place-conditioning study (sex effect: $F(1,57) = 4.81$, $p = 0.03$; PPA injection number effect: $F(9,513) = 385.65$, $p < 0.0001$; PPA × sex: $p = 0.60$; PPA × PPA injection number: $p = 0.91$; PPA × sex × PPA injection number: $p = 0.59$).

Protracted Effects of Pre-Adolescent PPA upon Cocaine-Induced Place Conditioning

Pre-adolescent PPA administration produced a dose-dependent enhancement in the expression of cocaine-induced place conditioning when mice were assessed in adulthood (Figure 2a). Whereas repeated saline administration did not elicit a change in the time spent in the nonpreferred compartment, the repeated pairing of 15 mg/kg cocaine with the nonpreferred compartment of the place-conditioning apparatus elicited an increase in the time spent in that compartment in all pretreatment groups (cocaine effect: $F(1,66) = 24.4$, $p < 0.0001$). As illustrated in Figure 2a, mice pretreated with 40 mg/kg PPA exhibited the greatest increase in the time spent in the cocaine-paired

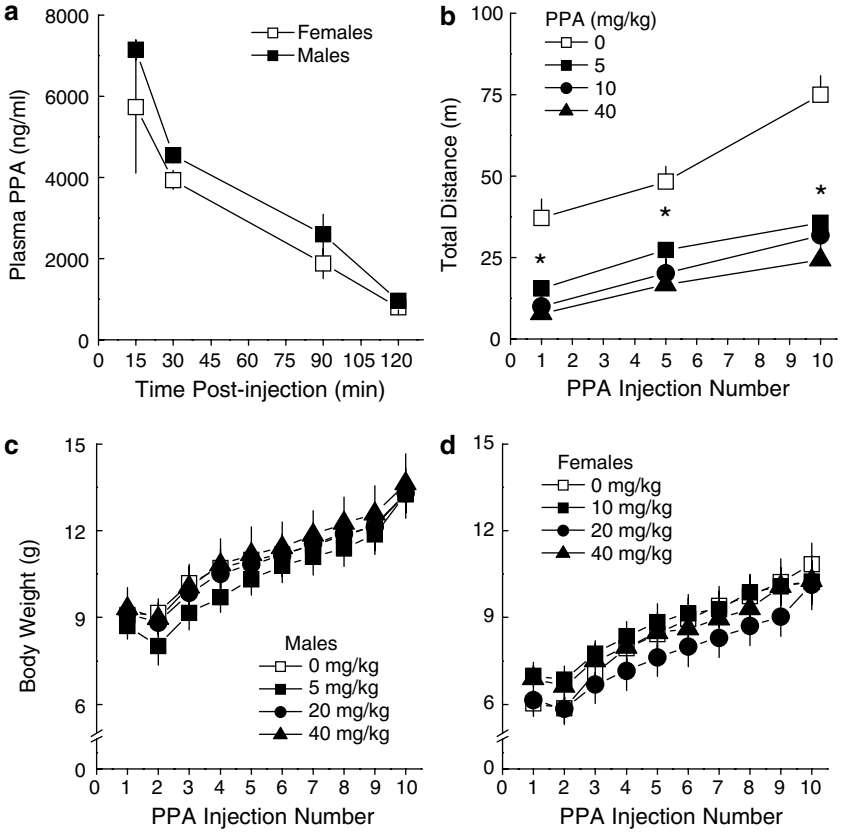

Figure 1 Effects of PPA administered during pre-adolescence. (a) The acute administration of 40 mg/kg PPA to PND 21 mice resulted in high plasma levels, which declined rapidly over sampling. The data represent the mean plasma concentrations of PPA ± SEM of 2–5 mice. (b) The repeated administration of PPA (0–40 mg/kg, i.p.) administered during PNDs 21–30 reduced locomotor activity in a dose-independent manner, compared to saline controls. The data represent the mean total distance traveled in a 2 h period ± SEM of 16–18 mice. Despite inducing psychomotor effects, pre-adolescent PPA administration did not influence the gain in bodyweight exhibited by either male (c) or female (d) pups. The data in (c) and (d) present the mean body weight (in g) ± SEM of 7–9 mice. *$p < 0.05$ *vs* SAL (0 mg/kg PPA).

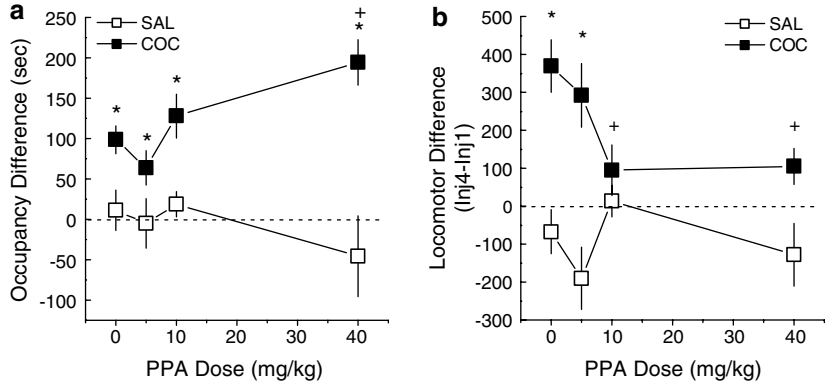

Figure 2 Enduring changes in cocaine behavioral responsiveness produced by pre-adolescent PPA. (a) Pre-adolescent PPA (0–40 mg/kg, i.p.) administered during PNDs 21–30 dose-dependently enhanced the amount of time adult mice spent in an environment paired previously with 15 mg/kg cocaine The data represent the mean difference in time spent in the nonpreferred compartment of the place-conditioning apparatus on the Pre-Test *vs* the Post-Test ± SEM of 6–8 animals. *$p < 0.05$ *vs* SAL (conditioning); +$p < 0.05$ *vs* 0 mg/kg PPA. (b) Pre-adolescent PPA produced a dose-dependent reduction in the expression of cocaine-induced locomotor sensitization induced by four injections of 15 mg/kg cocaine. The data represent the mean difference in the number of photocell mean breaks between injections one and four of repeated cocaine/saline treatment ± SEM of 6–8 animals. *$p < 0.05$ Inj 4 *vs* Inj1 (sensitization); +$p < 0.05$ *vs* 0 mg/kg PPA).

compartment following conditioning, compared to the other groups tested (PPA pretreatment effect: $F(3,66) = 1.42$, $p = 0.25$; PPA × cocaine: $F(3,66) = 4.82$, $p = 0.008$). Moreover, planned comparison of the time spent in the cocaine-paired *vs* the -unpaired compartment on the Post-Test revealed a significant place preference in the

cocaine-conditioned mice pretreated with 40 mg/kg PPA, which was not apparent in the other pretreatment groups (Table 1). The protracted effects of pre-adolescent PPA administration upon cocaine-induced place conditioning did not relate to effects of pretreatment upon the time spent in the nonpreferred chamber at the outset of conditioning, as the four treatment groups did not differ significantly on this measure (Figure 3a) (PPA pretreatment effect: $F(3,68) = 0.84$, $p = 0.48$).

Protracted Effects of Pre-Adolescent PPA upon Cocaine-Induced Locomotor Activity

Pre-adolescent PPA administration did not influence the acute locomotor response to 15 mg/kg cocaine when the mice were assessed at 10 weeks of age (PPA pretreatment × cocaine: $F(3,68) = 1.32$, $p = 0.26$; data not shown). However, pre-adolescent PPA administration dose-dependently blocked the development of cocaine-induced locomotor sensitization produced by the repeated administration of 15 mg/kg cocaine (Figure 2b). Whereas Repeated cocaine elicited a significant increase in the locomotor response to cocaine in mice pretreated with saline vehicle and 5 mg/kg PPA, whereas no such increase was apparent in mice pretreated with 10 or 40 mg/kg PPA (cocaine effect: $F(1,68) = 47.73$, $p < 0.0001$; PPA pretreatment effect: $F(3,68) = 2.59$, $p = 0.06$; PPA pretreatment × cocaine: $F(3,68) = 5.11$, $p = 0.003$).

Table 1 Summary of the Mean Time Spent (±SEM) in the Cocaine-Paired and the -Unpaired Compartments on the Post-Test

Pretreatment (mg/kg)	SAL-conditioned		COC-conditioned	
PPA	**Unpaired**	**Paired**	**Unpaired**	**Paired**
0	498.5±34.6	401.4±34.6	431.7±15.4	468.3±15.4
5	537.3±41.8	362.8±41.8	521.0±37.6	379.0±37.6
10	539.9±38.1	360.1±38.1	438.8±20.0	461.3 ±20.0
40	510.3±45.8	389.8±45.8	379.9±40.1	520.1±40.1*

SAL = saline; COC = 15 mg/kg cocaine. *$p < 0.05$ *vs* unpaired, indicating a place preference.

The protracted effects of pre-adolescent PPA administration upon cocaine-induced locomotor sensitization did not relate to effects of PPA upon measures of undrugged locomotor activity. As illustrated in Figure 2b, PPA pretreatment produced no observable effect upon the locomotor behavior of saline-paired mice (one-way ANOVA, $F(3,36) = 0.76$, $p = 0.52$). Moreover, PPA administration did not alter the locomotor response to being placed into the novel place-conditioning apparatus (Figure 3b) ($F(3,68) = 0.53$, $p = 0.66$), nor did it alter the locomotor response to the first saline injection (Figure 3c) ($F(3,68) = 0.33$, $p = 0.80$).

Protracted Effects of Pre-Adolescent PPA upon Cocaine-Induced Stereotypy

The reduction in the expression of cocaine-induced locomotor sensitization exhibited by PPA-pretreated mice did not relate to an increase in the expression of cocaine-induced stereotypy (Figure 4). As depicted in Figure 4a, a challenge injection of 15 mg/kg cocaine elicited the expression of locomotor sensitization in control mice treated thrice prior with cocaine in the home cage (SAL-COC), compared to mice injected acutely with cocaine (SAL-SAL), indicating sensitization. However, PPA pretreated mice injected thrice with 15 mg/kg cocaine in the home cage and then tested for cocaine-induced locomotion in the activity monitors (PPA-COC) did not differ from either group injected acutely with cocaine (Figure 4a) (PPA pretreatment effect: $F(1,28) = 46.9$, $p < 0.0001$; cocaine effect: $F(1,28) = 56.9$, $p < 0.0001$; PPA pretreatment × cocaine $F(1,28) = 31.9$, $p < 0.0001$). An examination of the stereotypy data indicated that whereas repeated cocaine administration induced a sensitization of stereotyped behaviors (Figure 4b), the expression of stereotypy was not affected by prior PPA experience (PPA pretreatment effect: $p = 0.08$; cocaine effect: $F(1,28) = 121.53$, $p < 0.0001$; PPA pretreatment × cocaine: $p = 0.193$).

Pre-Adolescent PPA Pretreatment Abolishes the Catecholamine Response to Cocaine in Adulthood

Cocaine is a monoamine reuptake inhibitor (Koe, 1976) and both the psychomotor-activating and rewarding properties of this stimulant are putatively mediated by an enhance-

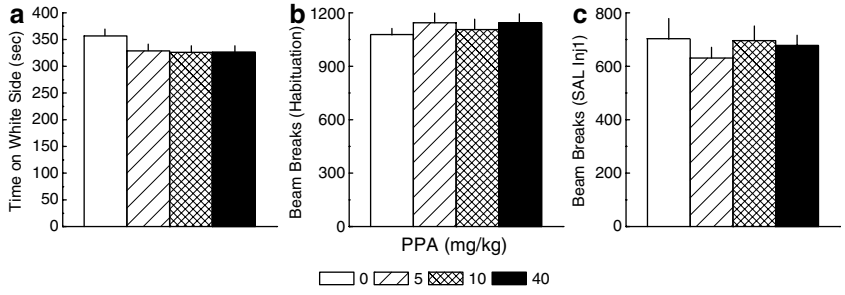

Figure 3 Pre-adolescent PPA exposure does not alter anxiety or spontaneous activity in adulthood. When assessed at 10 weeks of age, there was no observable effect of pre-adolescent treatment with PPA (0–40 mg/kg) regarding (a) the amount of time mice spent in the white compartment of the place-conditioning apparatus, (b) the locomotor activity exhibited by the animals in response to being placed in the place-conditioning apparatus for the first time or (c) the locomotor activity exhibited by the mice in response to their first saline (SAL) injection. The data represent the mean ± SEM of 14–18 animals.

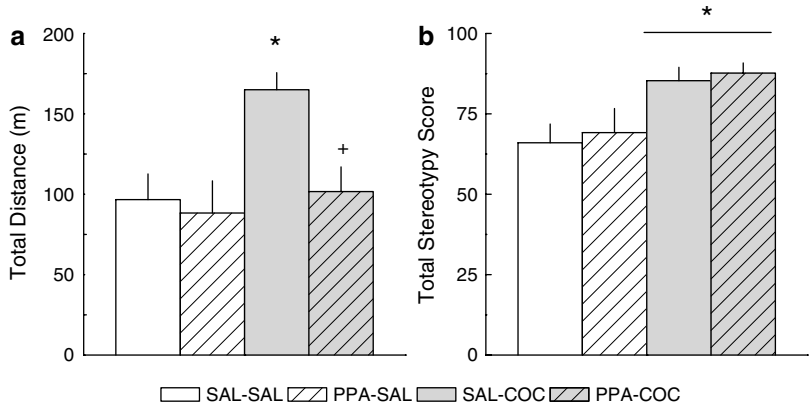

Figure 4 Differential effects of pre-adolescent PPA upon cocaine-induced locomotion and stereotypy. (a) Pre-adolescent PPA (0 or 40 mg/kg; i.p.) administered during PNDs 21–30 blocked the expression of locomotor sensitization when assessed in an unpaired testing environment. (b) Pre-adolescent PPA failed to alter the expression of stereotypy in either acute cocaine-treated or repeated cocaine-treated mice. The data represent the mean ± SEM of 7–8 animals. *$p<0.05$ *vs* repeated saline-treated mice (sensitization); +$p<0.05$ *vs* 0 mg/kg PPA.

ment in monoamine neurotransmission within the NAC (eg, Vanderschuren and Kalivas, 2000; Di Chiara *et al*, 2004; Hall *et al*, 2004). Thus, *in vivo* microdialysis was conducted in the NAC to relate the protracted behavioral effects of pre-adolescent PPA administration to alterations in the dopamine, norepinephrine and serotonin responses to acute and repeated cocaine. As summarized in Table 2, pre-adolescent PPA administration (40 mg/kg during PNDs 21–30) did not affect the average basal extracellular levels of any of the monoamines in the NAC (no main effects of, or interactions with, the PPA pretreatment factor: $p>0.05$). Despite this, a marked effect of pre-adolescent PPA administration was observed for the capacity of 15 mg/kg cocaine to elevate NAC extracellular levels of catecholamines (Figure 5). Whereas an acute injection of 15 mg/kg cocaine to control mice elevated extracellular levels of all three monoamines, the subchronic cocaine injection regimen induced a modest sensitization of this effect for dopamine (Figure 5a) and norepinephrine (Figure 5b), but not for serotonin (Figure 5c) (for dopamine, time effect: $F(11,220)=9.94$, $p<0.0001$; cocaine injection number × time: $F(11,220)=1.63$, $p=0.05$; for norepinephrine, time effect: $F(11,220)=5.85$, $p<0.0001$; cocaine injection number × time: $F(11,220)=2.51$, $p=0.005$; for serotonin, time effect: $F(11,220)=4.23$, $p<0.0001$; cocaine injection number × time: $F(11,220)=2.08$, $p=0.02$). As illustrated in Figure 5c, pre-adolescent PPA administration did not alter the modest cocaine-induced rise in extracellular levels of serotonin (PPA pretreatment effect: $p=0.78$; PPA pretreatment × time: $p=0.48$; PPA × cocaine injection number × time: $p=0.69$). However, 15 mg/kg cocaine failed to elicit a rise in extracellular dopamine (Figure 5a) and norepinephrine (Figure 5b) in the NAC of PPA-pretreated animals during either microdialysis session (for dopamine, PPA pretreatment effect: $F(1,20)=11.52$, $p=0.003$; PPA pretreatment × time: $F(11,220)=5.89$, $p<0.0001$; PPA pretreatment × cocaine injection number × time: $p=0.26$; for norepinephrine, PPA pretreatment effect: $F(1,20)=12.77$, $p=0.002$; PPA pretreatment × time: $F(11,220)=3.25$, $p<0.0001$; PPA pretreatment × cocaine injection number × time: $F(11,220)=2.84$, $p=0.002$).

Table 2 Summary of the Basal Extracellular Levels of Monoamines and Amino Acids in the NAC of Saline- and PPA-Pretreated Mice before Injections 1 and 4 of 15 mg/kg Cocaine as Derived Using Conventional *In Vivo* Microdialysis

Neurotransmitter content	SAL-pretreated		40 mg/kg PPA-pretreated	
	Inj1	Inj4	Inj1	Inj4
Dopamine (pg/sample)	12.9 ± 2.5	12.8 ± 1.3	15.1 ± 2.3	13.7 ± 1.7
Norepinephrine (pg/sample)	17.2 ± 6.7	13.7 ± 1.2	12.2 ± 6.3	13.5 ± 3.2
Serotonin (pg/sample)	20.3 ± 5.3	19.4 ± 1.7	24.3 ± 3.6	23.7 ± 3.2
Glutamate (ng/sample)	25.3 ± 8.5	22.4 ± 3.8	25.0 ± 4.8	20.6 ± 2.9
GABA (ng/sample)	2.2 ± 0.7	1.8 ± 0.2	1.6 ± 0.5	1.0 ± 0.1

Data represent the mean of three 20-min samples ± SEM of six to seven mice.

Pre-Adolescent PPA Pretreatment Facilitates Cocaine-Induced Adaptations in Amino-Acid Neurotransmission

A drug-induced imbalance in excitatory and inhibitory neurotransmission within the mesocorticolimbic circuit is a neural pathology implicated in the development of addiction-related behaviors (eg, Kalivas *et al*, 2005). Thus, we also examined for protracted effects of pre-adolescent PPA administration upon the glutamate and GABA responses to cocaine in the NAC (Figure 6). As summarized in Table 2, pre-adolescent PPA administration (40 mg/kg during PNDs 21–30) did not affect the average basal extracellular levels of either glutamate or GABA in the NAC (no main effects of, or interactions with, the PPA pretreatment factor: $p>0.05$). Consistent with previous studies in rodents (eg, Pierce *et al*, 1996; Szumlinski *et al*, 2004a, b, 2005b, 2006), an acute injection of 15 mg/kg cocaine to either group of mice did not affect NAC extracellular levels of glutamate (Figure 6a); however, following repeated cocaine administration, cocaine elicited a significant increase in glutamate above baseline in control mice (time effect: $F(11,253)=1.79$,

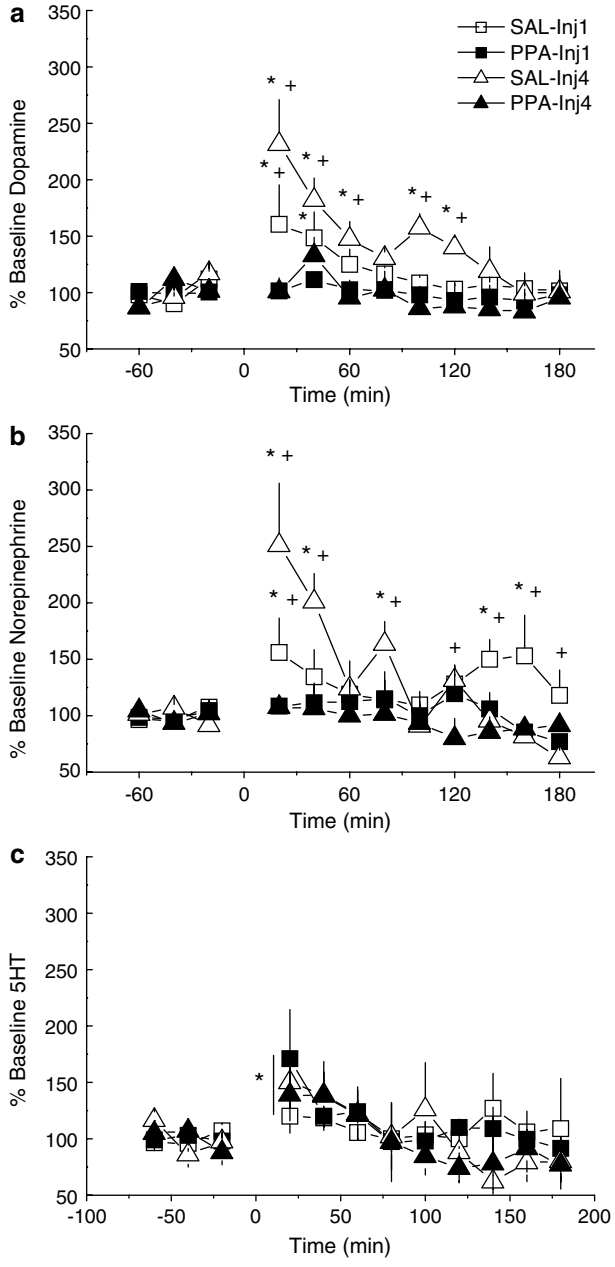

Figure 5 Enduring changes in the NAC catecholamine response to cocaine produced by pre-adolescent PPA. An acute injection of 15 mg/kg cocaine causes a sensitized rise in extracellular levels of catecholamines in saline (SAL)-pretreated animals, whereas pre-adolescent PPA administration (40 mg/kg) during PNDs 21–30 prevented the increase in extracellular dopamine (a) and norepinephrine (b) produced by cocaine (one or four injections of 15 mg/kg). (c) In contrast, PPA pre-exposure did not affect the capacity of 15 mg/kg to elevate NAC levels of serotonin. Data represent the mean percent change from baseline ± SEM of 6–8 animals. *$p<0.05$ *vs* Inj 1 (sensitization); +$p<0.05$ *vs* SAL (0 mg/kg PPA).

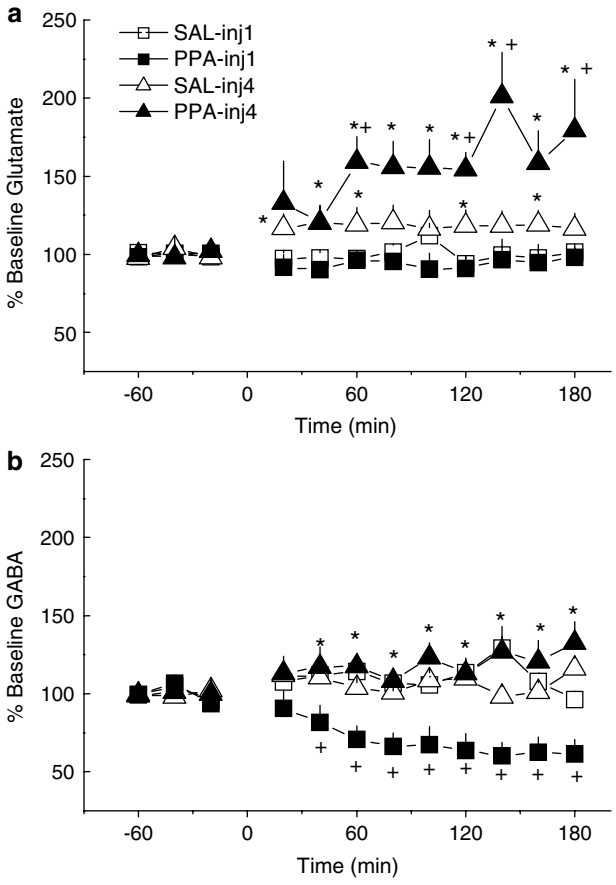

Figure 6 Enduring changes in the NAC amino acid response to cocaine produced by pre-adolescent PPA. (a) Whereas pre-adolescent PPA administration (40 mg/kg) during PNDs 21–30 did not alter the acute glutamate response to 15 mg/kg cocaine, it facilitated the development of cocaine-induced glutamate sensitization produced by four injections of 15 mg/kg cocaine. (b) Neither acute nor repeated cocaine altered extracellular GABA in saline (SAL)-pretreated mice. Acute cocaine lowered extracellular GABA below baseline in PPA-pretreated mice and this effect showed tolerance with repeated cocaine administration. Data represent the mean percent change from baseline ± SEM of 6–8 animals. *$p<0.05$ *vs* Inj 1 (sensitization); +$p<0.05$ *vs* SAL (0 mg/kg PPA).

$p=0.06$; time × cocaine injection number: $F(11,253)=3.20$, $p<0.0001$). As depicted in Figure 6a, the cocaine-sensitized increase in extracellular glutamate was significantly greater in mice pretreated with PPA (PPA pretreatment effect: $p=0.70$; PPA × time: $p=0.60$; PPA pretreatment × cocaine injection number × time: $F(11,253)=1.84$, $p=0.04$). As illustrated in Figure 6b, control animals did not exhibit a change in extracellular GABA following either acute or repeated cocaine (time effect: $p=0.78$; cocaine injection number × time: $F(11,253)=2.77$, $p=0.002$); however, PPA pretreated mice exhibited a large reduction in extracellular levels of GABA following the acute cocaine injection and this effect showed tolerance with repeated cocaine administration (PPA pretreatment effect: 0.29; PPA pretreatment × time: $F(11,253)=2.41$, $p=0.007$; PPA pretreatment × cocaine injection number × time: $F(11,253)=4.00$, $p<0.0001$).

DISCUSSION

For nearly 50 years, PPA was an active ingredient commonly found in popular children's OTC cough, cold, and allergy medicines. As PPA is a sympathomimetic (Hoffman, 2001), which can substitute for illicit stimulant drugs in self-administration and drug discrimination

studies (Woolverton *et al*, 1986; Chait *et al*, 1988; Lee *et al*, 1989; Wellman *et al*, 1989), the present study sought to determine whether exposure to PPA during the juvenile period of development produced protracted effects upon the behavioral and neurochemical effects of cocaine in adulthood. Here, we show that PPA exposure during the pre-adolescent period (PND 21–30) dose-dependently enhanced cocaine-conditioned reward and reduced cocaine-induced locomotor sensitization in mice (Figures 2 and 4). The protracted behavioral effects of pre-adolescent PPA exposure were accompanied by an altered dopamine, norepinephrine, glutamate, and GABA response to cocaine when assessed in adulthood. Pre-adolescent PPA exposure abolished the catecholamine response to cocaine in the NAC (Figure 5), facilitated the development of cocaine-induced glutamate sensitization (Figure 6a) and altered the GABA response to cocaine (Figure 6b). Despite these observed behavioral and neurochemical interactions between PPA and cocaine, pre-adolescent PPA produced no observable effects upon body weight during pre-adolescence (Figure 1) nor did it affect measures of basal anxiety, spontaneous locomotion or cocaine-induced stereotypy in adulthood (Figures 2–4). These data provide the first evidence that repeated exposure to PPA during pre-adolescence induces enduring neuroadaptations in NAC neurotransmission that may increase vulnerability to cocaine reward in adulthood.

PPA Effects during Pre-Adolescence

The systemic administration of PPA (40 mg/kg) produced a rapid elevation in plasma levels of PPA and the half-life of the drug in the plasma of juvenile mice following i.p. injection was estimated to be approximately 1.5–2 h (Figure 1a). Although sympathomimetic drugs can exert psychomotor-activating effects (eg, Eisenberg *et al*, 1987; Miller *et al*, 1999; Mittleman *et al*, 1993), earlier reports for PPA conducted in adult rats failed to observe hyperactivity in response to systemic injections of PPA (Eisenberg *et al*, 1987; Mittleman *et al*, 1993). As spontaneous locomotion, as well as drug-induced changes in motor hyperactivity, can depend upon the age of animal (eg, Bolanos *et al*, 1998; Carlezon and Konradi, 2004; Dafny and Yang, 2006; Niculescu *et al*, 2005; Spear and Brake, 1983), we examined for changes in the locomotor activity of juvenile mice in response to repeated PPA administration. Consistent with earlier reports for the locomotor effects of PPA in adult rats (Eisenberg *et al*, 1987; Mittleman *et al*, 1993), PPA administration to juvenile mice did not elicit motor hyperactivity, but rather suppressed locomotion in a dose-independent manner, relative to saline controls (Figure 1b). The earlier report by Mittleman *et al* (1993) implicated the locomotor-suppressive effects of even low doses of PPA (ie, 5 mg/kg) in the anorectic properties of this drug. However, we failed to detect any significant effect of PPA administration on body weight gain during PPA administration (Figure 1c and d) and the body weight of adult mice during the cocaine studies did not vary as a function of prior PPA treatment (data not shown).

In PPA-treated juvenile mice, we observed an increase in the expression of locomotor activity across PPA injections, indicative of sensitization. However, a similar increase in locomotor activity was observed also in control mice injected repeatedly with saline (Figure 1b). In our hands, the repeated administration of saline to adult mice typically induces either no change, but more often, a reduction in locomotion across injections, indicative of habituation to the environment, handling, and injection procedures (see Figure 2b; Szumlinski *et al*, 2002, 2004a, 2005a–c). Whether the change in locomotor behavior of saline controls reflected an age-dependent general increase in locomotor hyperactivity (eg, Niculescu *et al*, 2005; Spear and Brake, 1983) or a sensitized response to the injection stressor cannot be discerned by the results of the present study. However, in support of the latter possibility, the locomotor response to an acute saline injection does not differ between mice aged 21 and 28 days (Guerriero *et al*, 2006). Nonetheless, our data clearly demonstrate that PPA administration suppresses, rather than enhances, locomotor activity in juvenile mice. Thus, although PPA shares discriminative stimulus properties with illicit stimulant drugs (eg, Lee *et al*, 1989), it does not possess motor-activating properties in either adult or juvenile mice (eg, Eisenberg *et al*, 1987; Mittleman *et al*, 1993; present study).

Juvenile Licit Drug Exposure and Stimulant Addiction-Related Behaviors in Adulthood

The marked rise in the prescription of psychotropic drugs to children (Zito *et al*, 2000) has raised significant concern over the long-term psychobiological effects of prescription drug exposure during periods of brain development (cf., Carlezon and Konradi, 2004). In addition to its prevalence as the active ingredient in children's cough, cold, and allergy medications, PPA is also a major metabolite of methylphenidate (eg, Patrick *et al*, 1981)—the most highly prescribed medicine for the treatment of Attention-Deficit/Hyperactivity Disorder in children (eg, Zito *et al*, 2000). The present data for PPA are consistent with the notion that exposure to licit psychotropic drugs during periods of brain development produces enduring biobehavioral adaptations that persist into adulthood and extend these earlier findings for the prescription drug methylphenidate (cf., Carlezon and Konradi, 2004) to a formerly common OTC sympathomimetic. However, in contrast to earlier reports for methylphenidate (Andersen *et al*, 2002; Biederman *et al*, 1999; Carlezon *et al*, 2003; Mague *et al*, 2005; Torres-Reveron and Dow-Edwards, 2005), juvenile exposure to PPA enhanced the capacity of cocaine to elicit place conditioning in adulthood, but blocked the expression of cocaine-induced locomotor sensitization in both cocaine-paired and -unpaired environments (Figures 2 and 4).

The precise psychological underpinnings mediating the effects of juvenile PPA exposure upon cocaine-conditioned reward are unclear. A full dose–response characterization of the interactions between juvenile PPA administration and cocaine is required. However the observation that PPA pretreatment enhanced cocaine-conditioned reward, but prevented the development of cocaine-induced locomotor sensitization without influencing the expression of cocaine-induced stereotypy, suggests that juvenile PPA exposure produced some general increase in behavioral sensitivity to cocaine. Cocaine is anxiogenic in both humans and laboratory animals and withdrawal from repeated cocaine

administration induces a negative affective state (eg, Cohen, 1972; Covington and Miczek, 2003; Deroche-Gamonet *et al*, 2004; Gawin and Kleber, 1986; Gawin, 1991; Gillman *et al*, 2006; Jung *et al*, 2002; Markou and Koob, 1991; McGregor *et al*, 2005; Paine *et al*, 2002). Moreover, the aversive properties of cocaine can become conditioned to the environment in which the drug is repeatedly administered (eg, Ettenberg and Geist, 1991; Raven *et al*, 2000). Indeed, studies of both humans and laboratory animals have implicated an enhancement of cocaine-induced psychomotor activation (notably stereotypies) and aversion or an increase in anhedonia as mechanisms to account for the observed 'protective' effect against subsequent cocaine addiction-related behaviors in adulthood engendered by juvenile exposure to methylphenidate (Andersen *et al*, 2002; Biederman *et al*, 1999; Carlezon *et al*, 2003; Mague *et al*, 2005; Torres-Reveron and Dow-Edwards, 2005). Yet, our interactions between juvenile PPA treatment and cocaine administration in adulthood are distinct, if not opposite, to those observed previously for methylphenidate (eg, Andersen *et al*, 2002; Augustyniak *et al*, 2006; Carlezon *et al*, 2003; Torres-Reveron and Dow-Edwards, 2005). Thus, perhaps a converse mechanism may be invoked to account for our data; pre-adolescent PPA administration may facilitate the development of cocaine-conditioned reward by reducing either the unconditioned or the conditioned aversive effects of cocaine. Although this mechanism is under intense investigation by our laboratory, our present observation that juvenile PPA treatment prevented the development of cocaine-induced locomotor sensitization, a classic animal model of psychosis (eg, Scheel-Kruger *et al*, 1977; Kuczenski *et al*, 1991; Post and Kopanda, 1976; Segal and Kuczenski, 1997), is consistent with this hypothesis.

In addition to positive and negative rewarding properties, psychomotor-stimulant drugs have behavioral disinhibitory properties (Olausson *et al*, 2000; Spinella, 2003; Szumlinski *et al*, 2000a). Thus, it may be postulated that juvenile PPA exposure increased the time spent on originally nonpreferred, cocaine-paired, side by facilitating behavioral disinhibition. However, two observations argue against a major role for changes in behavioral disinhibition in the observed facilitation of cocaine-induced place conditioning by juvenile PPA exposure. First, when assessed for compartment preference at the outset of place conditioning, the large majority of animals avoided the white compartment of the apparatus and significant pretreatment differences were not observed in this regard (Figure 3a). Moreover, PPA pretreatment reduced locomotor activity during pre-adolescence (Figure 1), whereas it failed to produce a protracted effect upon the expression of locomotor activity elicited by either placement into a novel environment (Pre-Test1) or in response to a saline injection (Figure 3). Juvenile PPA treatment produced no observable protracted effects upon these measures of anxiety/behavioral disinhibition. Also, the facilitation of time spent in the originally non-preferred compartment by PPA-pretreated mice was observed in the absence of cocaine and thus, cannot be attributed to a change in the behavioral disinhibitory properties of cocaine *per se*. As a biased place-conditioning procedure was employed in the present study, we cannot negate a role for other nonreward mechanisms to account for the increase in the time spent in the cocaine-paired environment exhibited by PPA-pretreated mice. However, for the reasons presented above, we argue that an attenuation of the psychomotor-sensitizing properties of cocaine is a plausible explanation to account for the apparent 'pro-addictive' effects of juvenile PPA exposure.

Pre-Adolescent PPA Produces Persistent Perturbations in NAC Extracellular Catecholamine Levels

Cocaine, methylphenidate, and PPA all block the reuptake of norepinephrine (Hoffman, 2001; Koe, 1976; Solanto, 1998); however, PPA differs from both cocaine and methylphenidate in that it does not prevent dopamine reuptake, but acts as a direct, nonselective adrenergic receptor agonist (Hoffman, 2001). Thus, we reasoned that the protracted effects of PPA vis-à-vis cocaine-induced changes in behavior in adulthood might reflect selective perturbations in noradrenergic transmission that, in turn, may alter the activity of brain regions involved in drug reward and locomotor hyperactivity, such as the NAC. Few studies have examined the neurochemical effects of PPA administration in laboratory animals. PPA produces only a modest effect upon dopamine release in striatal synaptosomal preparations, it enhances both dopamine and norepinephrine release in cortical preparations (Baldessarini and Harris, 1974). An examination of the effects of repeated PPA administration upon tissue content of dopamine revealed a selective reduction in tissue content of dopamine in the prefrontal cortex, but not in the NAC of rhesus monkeys (Woolverton *et al*, 1986). Consistent with this latter observation, we failed to detect any observable protracted effect of pre-adolescent PPA exposure (10×40 mg/kg) upon basal extracellular levels of monoamines in the NAC. However, we observed a complete blockade of the capacity of both acute and repeated cocaine to elevate extracellular levels of dopamine and norepinephrine in the NAC of adult mice (Figure 5a and b). Interestingly, this protracted PPA effect did not generalize to all monoamines, as the serotonin response to cocaine was unaffected (Figure 5c). As cocaine is a monoamine reuptake inhibitor, the capacity of cocaine to elevate extracellular levels of monoamines in terminal regions depends upon its binding to cell-surface monoamine transporter proteins and upon the release of these neurotransmitters into the synaptic cleft (Koe, 1976). In the NAC, norepinephrine and dopamine release is inhibited by $\alpha 2$ adrenergic receptors localized on norepinephrine and dopamine terminals (Ihalainen and Tanila, 2004). Thus, we postulate two potential mechanisms to account for our observed catecholamine effects of juvenile PPA exposure. First, pre-adolescent PPA may produce a persistent perturbation in NAC catecholamine release via a sensitization of $\alpha 2$ adrenergic receptor function. Second, pre-adolescent PPA may upregulate the expression or function of the dopamine and norepinephrine transporters, necessitating the administration of higher doses of cocaine to elicit a rise in extracellular levels of these neurotransmitters. Studies have commenced in the laboratory to investigate these possibilities and preliminary data support a potential role for both mechanisms in mediating the blunted catecholamine response to cocaine produced by pre-

adolescent PPA exposure (Szumlinski, Ary, Renton, and Tieu, unpublished data).

The behavioral relevance of the blunted NAC catecholamine response to cocaine in PPA-pretreated mice is not clear at the present time. Dopamine, and to a lesser extent norepinephrine, have been implicated in mediating drug reward, and in particular, drug reward-related learning (eg, Di Chiara, 1999; Everitt *et al*, 1999; Robinson and Berridge, 2001). However, the PPA-induced facilitation of cocaine-conditioned reward in adulthood was associated with a reduction in, not an enhancement of, the catecholamine response to cocaine. Both norepinephrine and dopamine are implicated also in the psychomotor-activating/psychogenic effects of stimulant drugs (Sherer, 1988). Yet, despite profound effects of juvenile PPA treatment upon the catecholamine response to acute cocaine (Figure 5), we failed to observe group differences in the acute locomotor or stereotypic response to cocaine in adulthood (Figure 4). The only observed effect of pre-adolescent PPA administration that is consistent with a blunted catecholamine system is the attenuation of locomotor sensitization. However, two major lines of evidence argue against a direct relationship between the protracted effects of juvenile PPA exposure and the locomotor- and catecholamine-sensitizing effects of cocaine. For one, the locomotor-activating or -sensitizing effects of psychomotor stimulants can be dissociated from the ability of this drug class to elevate extracellular levels of dopamine and norepinephrine in the NAC (eg, Frantz *et al*, 2006; Ito *et al*, 2006; Szumlinski *et al*, 2000a, b, 2004b, 2005b; Zhang *et al*, 2006). These observations are supported by the present finding that despite reducing the capacity of acute cocaine to elevate NAC levels of dopamine and norepinephrine, PPA pretreatment did not alter the acute motor-stimulatory effects of cocaine. Second, considerable behavioral pharmacological evidence demonstrates that dopamine or norepinephrine receptor activation is not necessary for the development of cocaine-induced behavioral sensitization (eg, Szumlinski *et al*, 2004b; Thiébot *et al*, 1981; Vanderschuren *et al*, 2003; see also review by Vanderschuren and Kalivas, 2000). Thus, it is not likely that a causal relationship exists between the deficit in catecholamine responsiveness to cocaine and the lack of behavioral sensitization observed in PPA pretreated mice in the present study.

Pre-Adolescent PPA Facilitates Cocaine-Induced Plasticity in NAC Glutamate Transmission

Cocaine-induced sensitization of glutamate release within the NAC is a putative neural substrate for both the addictive and motor-sensitizing properties of cocaine (Pierce *et al*, 1996; Baker *et al*, 2003; Szumlinski *et al*, 2004a, b, 2006). Consistent with current glutamate theories of addiction (eg, Everitt and Wolf, 2002; Kalivas *et al*, 2005), the enhancement in cocaine-conditioned reward observed in PPA-pretreated mice was paralleled by significantly greater cocaine-induced glutamate sensitization in the NAC. Moreover, in control mice, the presence of cocaine-induced motor sensitization was accompanied by a sensitized NAC glutamate response to cocaine. However, the expression of sensitized locomotor behavior by PPA mice was inversely related to the extent to which repeated cocaine elicited glutamate sensitization in the NAC. Given that only one dose of cocaine was investigated in the present study, one might argue that cocaine may have induced the expression of focused, stereotyped behaviors in PPA-pretreated mice and index of increased sensitivity to cocaine. However, we failed to detect group differences in cocaine-induced stereotypy elicited by 15 mg/kg cocaine in either acute or repeated cocaine-treated mice (Figure 4). A second possibility to account for the apparent discrepancy between cocaine-induced locomotor and glutamate sensitization is that whereas glutamate sensitization in the NAC may be necessary for the development of cocaine-induced motor sensitization (Pierce *et al*, 1996), it is not sufficient to induce this behavioral change. As cocaine failed to elicit a rise in NAC extracellular levels of catecholamines in PPA-pretreated mice, it is plausible that adaptations in catecholamine and glutamate transmission must co-occur for the full expression of this form of cocaine-induced behavioral plasticity. Whereas the results of the present study cannot discern between these two possibilities, the present results nonetheless indicate that pre-adolescent PPA administration produces an enduring effect upon the cocaine responsiveness of glutamate terminals in the NAC. Whereas the precise mechanisms mediating the protracted effects of PPA administration upon cocaine-induced glutamate release in the NAC remain to be determined, adrenoreceptors regulate glutamate release within the prefrontal cortex (Golembiowska and Zylewska, 1999; Marek and Aghajanian, 1999). As our current working hypothesis is that the repeated stimulation of adrenoreceptors by PPA during pre-adolescence induces alterations in receptor function that endure into adulthood, future studies in our laboratory will be examining more closely also the role for these two adrenoreceptors in regulating cocaine-induced changes in glutamate transmission within the NAC as it pertains to the protracted effects of pre-adolescent PPA administration.

Pre-Adolescent PPA Alters the GABA Response to Cocaine

The majority of GABA released within the NAC is derived from neuronal sources (Xi *et al*, 2003), which include the small population of aspiny GABAergic interneurons, the extensive network of recurrent collaterals of medium spiny neurons and GABAergic afferents to the NAC (including the ventral pallidum, ventral tegmental area or olfactory nucleus) (Smith and Bolam, 1990; Brog *et al*, 1993; Pennartz *et al*, 1994). Withdrawal from repeated cocaine administration increases basal GABA content in the NAC and this neuroadaptation has been proposed to contribute to the dysregulation of NAC physiology following repeated cocaine experience (Xi *et al*, 2003). Despite now a decade of research concerned with the role for cocaine-induced alterations in NAC glutamate transmission in mediating the behavioral consequences of repeated stimulant administration (Everitt and Wolf, 2002; Kalivas *et al*, 2005), the effects of a cocaine injection upon extracellular GABA levels within the NAC are currently undescribed. In the present study, pre-adolescent PPA administration facilitated an inhibition of extracellular GABA following an acute cocaine injection. Moreover, the cocaine-induced reduction in extracellular

GABA observed in PPA-pretreated mice showed tolerance with repeated cocaine administration, with the fourth injection of cocaine elevating NAC extracellular GABA levels above baseline (ie, sensitization) (Figure 6b). A considerable amount of behavioral pharmacological data strongly implicate GABAergic neurotransmission in mediating the rewarding and psychomotor-activating effects of cocaine (eg, Brebner *et al*, 2002; Gardner *et al*, 2002; Itzhak and Martin, 2000; Roberts, 2005; Steketee and Beyer, 2005). However, despite the large difference in their GABA response to an acute cocaine injection, PPA-pretreated mice did not differ from controls in terms of their locomotor response to an acute injection of this drug. Furthermore, the subchronic cocaine regimen employed in the present study induced both locomotor sensitization and conditioned reward in control mice, yet did not alter levels of GABA in the NAC either basally or following a cocaine injection. Thus, whereas a history of cocaine experience may elevate NAC GABA content (Xi *et al*, 2003) and enhance GABA release in the ventral pallidum in a manner that correlates with sensitized lever pressing behavior (Tang *et al*, 2005), the precise relationship between our GABAergic findings in PPA-pretreated mice and behavioral and neurochemical responsiveness to cocaine remain unresolved.

CONCLUSIONS

Until December 2005, PPA was a common active ingredient found in popular children's cold, cough and allergy medicines. This work demonstrates for the first time that repeated administration of PPA to juvenile mice produces a protracted increase in cocaine reward, concomitant with a reduction in cocaine-induced locomotor sensitization. The enduring behavioral effects of early PPA exposure are associated with deficits in catecholaminergic transmission, as well as a facilitation of cocaine-induced sensitization of amino-acid transmission within the NAC. As PPA was available for 50 years in both prescription and OTC preparations and is a major metabolite of methylphenidate, a more intensive investigation into the protracted psychobiological consequences effects of early exposure to PPA, as well as to related and available OTC sympathomimetics (eg, pseudoephedrine), is warranted to gain a more solid understanding of the mechanism(s) through which childhood exposure to sympathomimetics may impact vulnerability to addictive and psychotic disorders in later life.

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