Available online at www.sciencedirect.com
SCIENCE DIRECT®
Pharmacology, Biochemistry and Behavior 82 (2005) 280 – 288
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Age-specific behavioral responses to psychostimulants in mice

Michelle Niculescu [a,*], Michelle E. Ehrlich [b], Ellen M. Unterwald [a,c]

[a] Department of Pharmacology and Center for Substance Abuse Research, Temple University School of Medicine, Philadelphia, PA 19140, United States
[b] Farber Institute for Neurosciences and Department of Neurology, Thomas Jefferson University College of Medicine, Philadelphia, PA 19107, United States
[c] Laboratory of the Biology of Addictive Diseases, The Rockefeller University, New York, NY 10021, United States

Received 19 January 2005; received in revised form 22 August 2005; accepted 23 August 2005
Available online 30 September 2005

Abstract

This study investigated the influence of age on the behavioral responses elicited by psychostimulants in male CD-1 mice. Behavioral activity including locomotion and stereotypy was measured following acute or repeated administration of cocaine, methylphenidate, amphetamine or saline to postweanling (24 days old), periadolescent (33 days old) and adult (60 days old) mice. Postweanling mice exhibited less total and ambulatory activity than periadolescent mice following a single acute injection of cocaine (20 or 30 and 30 mg/kg, respectively). Further, postweanling mice showed less total activity than both periadolescent and adult mice at a dose of 10 mg/kg methylphenidate. Less stereotypy was also seen in postweanling mice when compared to adolescent mice after 30 mg/kg amphetamine. Seven daily injections of cocaine resulted in a heightened behavioral response on day 7 as compared to day 1, indicative of behavioral sensitization in adult and periadolescent, but not postweanling mice. Repeated methylphenidate resulted in increased total activity in adult, but not periadolescent or postweanling mice. None of the animals were sensitized to the behavioral activating effects of amphetamine. The magnitude of behavioral response and the development of sensitization were dependent upon the age of the animal and the agent tested.

Keywords: Cocaine; Methylphenidate; Amphetamine; Mice; Sensitization; Development; Adolescent

1. Introduction

The use of psychomotor stimulants by children and adolescents continues to rise due to both the treatment of Attention Deficit Hyperactivity Disorder (ADHD) and illicit abuse (Cantwell, 1996). Responses to psychostimulants can vary with age. During adolescence and adulthood, stimulants elicit a euphoric response, while they have been described as dysphoric by children (Rapoport et al., 1980). Despite noted differences, few studies have systematically compared the effects of psychostimulants in mice of different ages. This has prompted our study of the effects of both acute and repeated exposure to these drugs in different age groups.

Cocaine, methylphenidate and amphetamine are psychomotor stimulants which all enhance synaptic neurotransmitter concentrations, but with somewhat different mechanisms of action. Cocaine inhibits the reuptake transporters for dopamine, serotonin and norepinephrine, thereby increasing these neurotransmitter concentrations in the synapse (Heikkila et al., 1975a). Methylphenidate also blocks dopamine and norepinephrine reuptake transporters causing an increase in extracellular levels, but exerts a minimal effect on serotonin transporters (Kuczenski et al., 1997). Amphetamine increases dopamine, norepineprine and serotonin in the synapse by increasing their release (Heikkila et al., 1975b; Connor and Kuczenski, 1986). Methylphenidate and amphetamine are used therapeutically to treat common childhood illnesses, whereas all three drugs are used illicitly by adolescents and adults.

Acutely, cocaine, amphetamine and methylphenidate administration can produce dose-dependent behavioral activation (Heffner and Seiden, 1982; Gerasimov et al., 2000; Schramm-Saptya et al., 2004). In adult animals, the repeated administration of psychostimulants can result in an augmentation of the behavioral responses. Behavioral sensitization, or an increased response to subsequent psychostimulant dosing after previous exposure, has been shown to develop to the locomotor-

* Corresponding author. 312 Medical Research Building, Temple University School of Medicine, Department of Pharmacology, 3420 N. Broad St., Philadelphia, PA 19140, United States. Tel.: +1 215 707 8818; fax: +1 215 707 7068.
E-mail address: michelleniculescu@yahoo.com (M. Niculescu).

0091-3057/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2005.08.014

stimulating effects of these drugs in rodents (Post and Rose, 1976; Shuster et al., 1982; Stewart and Badiani, 1993). Many neuronal systems including, but not limited to, dopamine, glutamate, serotonin and opioid are thought to be involved in the development of behavioral sensitization (Koe, 1976; Parsons and Justice, 1993; DiChiara, 1995; Wolf, 1998; Vanderschuren and Kalivas, 2000; Everitt and Wolf, 2002; Tzschentke and Schmidt, 2003; Hummel et al., 2004). Specifically, hypersensitization of mesocorticolimbic dopamine neurotransmission is thought to be an integral factor in this phenomenon and has also been linked to drug craving (Robinson and Berridge, 1993). Therefore, if behavioral sensitization is an age-dependent response to chronic psychostimulant administration, it may be indicative of differential sensitivities to the addictive potential of these drugs.

Distinct age-related responses to psychostimulants have been reported. For example, periadolescent rats show an attenuated locomotor response to acute administration of cocaine and amphetamine when compared to adults (Spear and Brake, 1983; Laviola et al., 1999; Adriani and Laviola, 2000). As measured by Cirulli and Laviola, postweanling mice show a heightened sensitivity to amphetamine-induced increases in locomotor and stereotypic activity when compared to preweanling mice (Cirulli and Laviola, 2000). However, it is difficult to generalize the findings following chronic administration of psychostimulants in different ages due to the inconsistencies in the reported results. While some reports show that both periadolescent and adult rodents sensitize to the locomotor-stimulating effects of cocaine, methylphenidate and amphetamine (Laviola et al., 1995; Adriani et al., 1998; McDougall et al., 1999; Shuster et al., 1982), others show that periadolescent rats do not sensitize to the locomotor-stimulating properties of cocaine (Collins and Izenwasser, 2002). Still another group showed sensitization to the locomotor-activating properties of cocaine in periadolescent, but not adult mice if there was no habituation period (Schramm-Saptya et al., 2004). Published data on the behavioral effects of psychostimulants are inconsistent probably due to the use of a variety of experimental paradigms, drug doses, routes of drug administration and strains and species of the animals tested. Therefore, a systematic study to compare the behavioral effects of cocaine, amphetamine and methylphenidate in different age animals was warranted.

Specifically, the present study characterized the acute behavioral effects of several doses of cocaine, methylphenidate and amphetamine, as well as the effects of repeated injections of these agents in postweanling, periadolescent and adult mice within identical treatment paradigms. Mice were chosen for these studies because of the widespread use of genetically engineered mice in neuroscience and addiction research.

2. Methods

2.1. Subjects-housing and treatment

Male CD-1 mice were obtained from Charles River Laboratories. Mice were housed four per Plexiglas cage ($28 \times 18 \times 14$ cm) in a temperature (21 ± 1 °C) and relative humidity ($40 \pm 10\%$)-controlled room and with a 12-h light/dark cycle (lights on at 7:00 a.m.). Animals were housed for five days prior to being tested and had free access to standard laboratory chow and tap water, except during activity monitoring. All experiments were conducted in accordance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals and with an approved protocol from Temple University School of Medicine Institutional Animal Care and Use Committee.

Animals of each age group, postweanling (24 days old), periadolescent (33 days old) and adult (60 days old), were assigned randomly to a cocaine, methylphenidate or amphetamine treatment group. Each treatment group had an age-matched saline-injected control group.

2.2. Drugs

Cocaine HCl and D-amphetamine, generously supplied by the National Institute of Drug Abuse (NIDA), and methylphenidate (Sigma, St. Louis, MO) were dissolved in sterile saline (0.9% NaCl). Drug or saline was administered intraperitoneally (IP) at a volume of 3 ml/kg body weight.

2.3. Behavioral testing

Activity was monitored in eight identical transparent Plexiglas boxes ($45 \times 20 \times 20$ cm) using Digiscan™ activity monitors (Accuscan, Columbus, OH). The monitors are equipped with 16 infrared light emitters and detectors. The number of times the photo beams were broken was cumulated by a microcomputer. The total number of beam breaks is reported herein as 'total activity', whereas 'ambulatory activity' includes only bouts of successive beam breaks and 'stereotypic counts' include only bouts of repetitive beam breaks. While stereotypic counts cannot directly measure the specific stereotypic activity, repeated breaks of the same beam indicate a stationary animal engaged in repetitive behavior as opposed to locomotion. Animals were placed in the monitors 30 min prior to drug or saline injections. Activity was monitored for 30 min after the injection. All behavioral testing was done between 1:00 and 5:00 p.m.

2.4. Drug administration

For acute drug administration, postweanling (24 days old), periadolescent (33 days old) and adult (60 days old) mice received a single injection of cocaine (0, 10, 20 or 30 mg/kg IP), methylphenidate (0, 5, 10 or 20 mg/kg IP) or amphetamine (0, 2.5, 5 or 10 mg/kg IP) and activity was measured for 30 min post-injection.

In a separate set of experiments, cocaine (20 mg/kg IP), methylphenidate (10 mg/kg IP), amphetamine (5 mg/kg IP) or saline (3 ml/kg IP) was administered to postweanling, periadolescent and adult mice once daily for seven days beginning on postnatal days 24, 33 and 60, respectively.

Activity was measured for 30 min post-injection on days 1 and 7 as described above.

2.5. Data analysis

Total, ambulatory and stereotypic activity from each age group was compared by two-way ANOVA with dose and age as factors, followed by Bonferonni post hoc test when significant. To test for sensitization, activities on days one and seven were compared by one-way ANOVA. Post hoc comparisons were performed using the Bonferonni if ANOVA was significant.

3. Results

3.1. Acute drug administration: stereotypy

There were no differences in the baseline stereotypy between the age groups. Acute cocaine administration resulted in dose-dependent increases in the expression of behavioral stereotypy in postweanling, periadolescent and adult mice. While 10 and 20 mg/kg cocaine produced similar levels of stereotypy in the three age groups of mice, postweanling mice showed significantly less stereotypic activity counts than periadolescent mice at a dose of 30 mg/kg as determined by two-way ANOVA [$F(2,83)=5.69$, Bonferonni post hoc comparison determined $P<0.05$] (Fig. 1A). Methylphenidate also increased stereotypic behavior in a dose-dependent manner in all the age groups. There were no differences in stereotypic counts between postweanlings, periadolescents or adults at 0 (baseline), 5, 10 or 20 mg/kg of methylphenidate (Fig. 1B). Acute amphetamine administration produced increases in stereotypic activity in all three age groups, but there were no differences between the age groups (Fig. 1C).

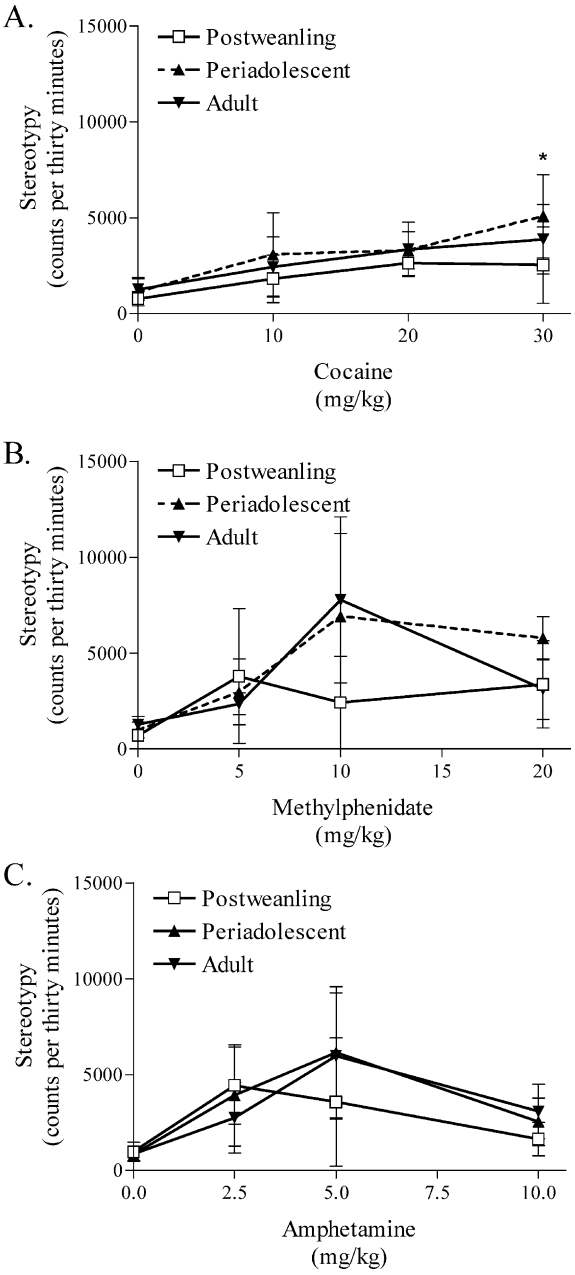

Fig. 1. Stereotypic counts for 30 min following a single (A) cocaine (0, 10, 20 or 30 mg/kg), (B) methylphenidate (0, 5, 10 or 20 mg/kg) or (C) amphetamine (0, 2.5, 5 or 10 mg/kg) (C) injection in postweanling, periadolescent and adult mice ($n=6-8$). Postweanling mice showed less stereotypic activity following an acute cocaine injection (30 mg/kg) than did periadolescent mice (*$P<0.05$).

3.2. Acute drug administration: ambulatory activity

No differences in baseline ambulatory activity between postweanling, periadolescent and adult mice were found. Acute administration of cocaine increased ambulatory activity in all three age groups. At a dose of 30 mg/kg of cocaine, two-way ANOVA determined that postweanling mice had significantly lower ambulatory activity than periadolescent mice [$F(2,84)=3.31$; Bonferonni post hoc comparison determined $P<0.05$] (Fig. 2A). Methylphenidate increased ambulatory activity in a dose-dependent manner, which did not differ between age groups (Fig. 2B). Acute administration of amphetamine also increased ambulatory activity in all the age groups, but there were no differences between the ages (Fig. 2C).

3.3. Acute drug administration: total activity

Cocaine produced an increase in total activity in postweanling, periadolescent and adult mice. At doses of 20 and 30 mg/kg of cocaine, postweanling mice had significantly lower total activity counts than periadolescent mice [$F(2,84)=6.29$; Bonferonni post hoc determined $P<0.05$ for both doses] (Fig. 3A). Methylphenidate (20 mg/kg) induced less total activity in postweanling mice when compared to periadolescent and adult mice as determined by two-way ANOVA [$F(2,60)=4.42$; Bonferonni post hoc test determined $P<0.05$] (Fig. 3B). Amphetamine (Fig. 3C) increased total activity as compared to saline-injected mice, but there were no differences in amphetamine-induced total activity between the age groups, although postweanling mice had the lowest levels of drug-induced activity.

3.4. Repeated drug administration: stereotypy

Postweanling, periadolescent and adult mice were injected with cocaine (20 mg/kg), methylphenidate (10 mg/kg),

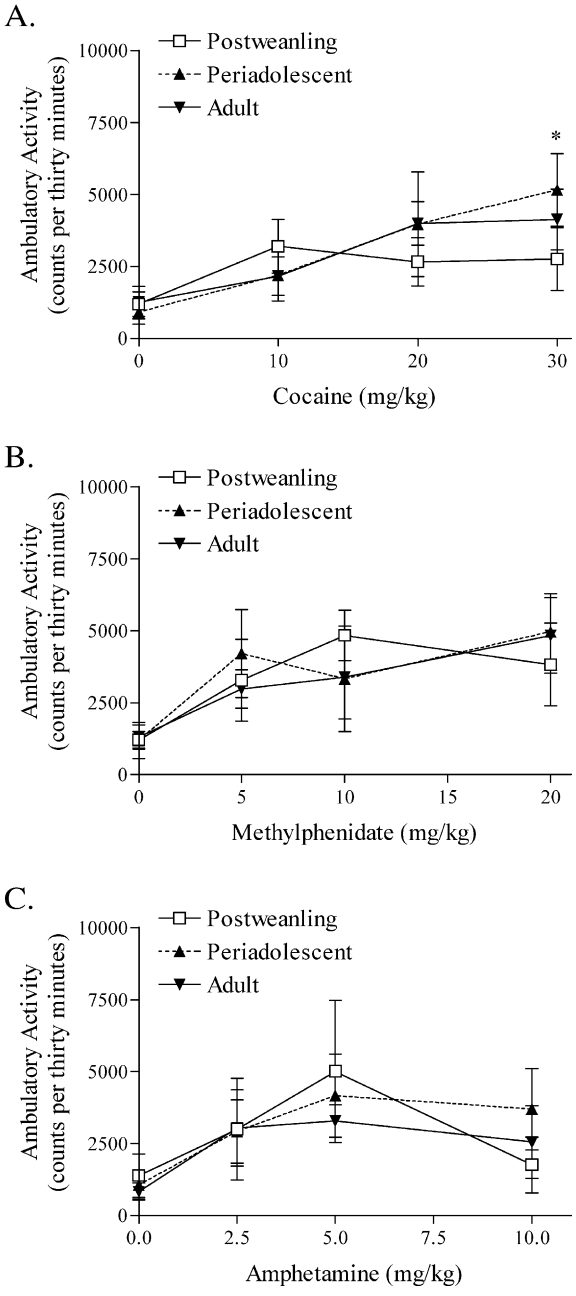

Fig. 2. Ambulatory activity for 30 min following a single (A) cocaine (0, 10, 20 or 30 mg/kg), (B) methylphenidate (0, 5, 10 or 20 mg/kg) or (C) amphetamine (0, 2.5, 5 or 10 mg/kg) (C) injection in postweanling, periadolescent and adult mice. Postweanling mice showed less ambulatory activity following an acute cocaine injection (30 mg/kg) than periadolescents (*$P<0.05$) ($n=6-8$).

amphetamine (5 mg/kg) or saline (3 ml/kg) once daily for seven days. Fig. 4 shows stereotypic counts on days one and seven of the study. On both test days in all of the age groups, cocaine-, methylphenidate- and amphetamine-injected mice had significantly more stereotypic counts than age-matched saline controls. Stereotypic counts following cocaine were significantly higher on day one compared to day seven by one-way ANOVA in adult mice [$F(5,47)=4.122$, $P<0.001$] (Fig. 4A). There were no differences in stereotypic counts between day one compared to day seven in any age group following methylphenidate (Fig. 4B) and amphetamine (Fig. 4C).

3.5. Repeated drug administration: ambulatory activity

Fig. 5 represents ambulatory activity following one and seven days of cocaine (20 mg/kg) (A), methylphenidate (10 mg/kg) (B), and amphetamine (5 mg/kg) (C). Psychostimulant administration increased ambulatory activity on days one and

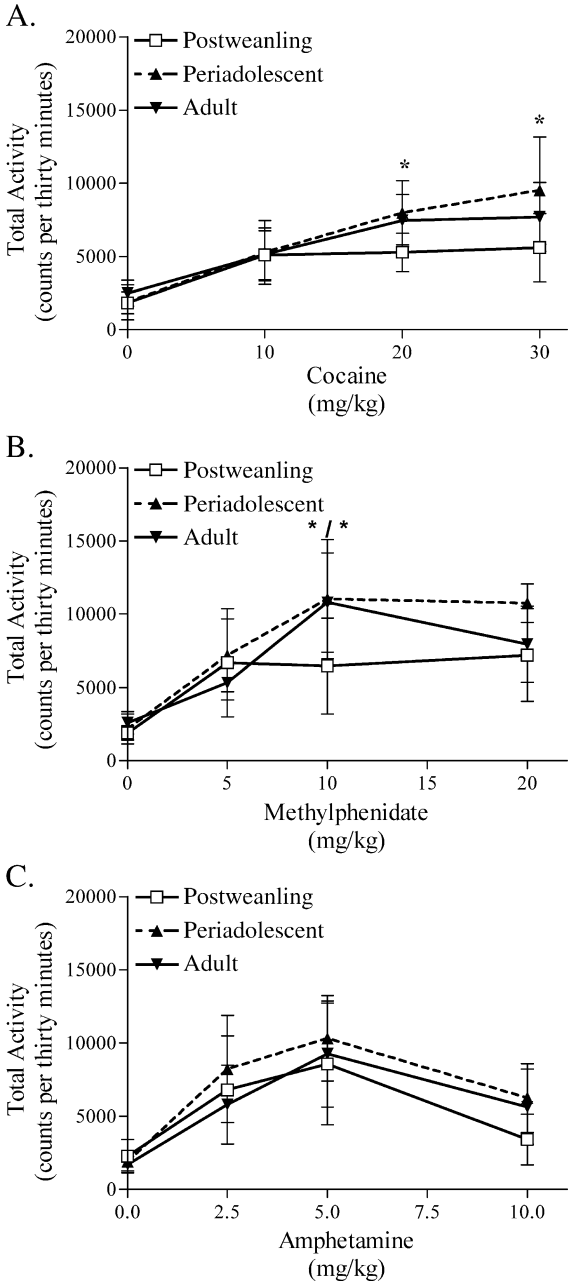

Fig. 3. Total activity in postweanling, periadolescent and adult mice following a single (A) cocaine (0, 10, 20 or 30 mg/kg), (B) methylphenidate (0, 5, 10 or 20 mg/kg) or (C) amphetamine (0, 2.5, 5 or 10 mg/kg) injection. Postweanling mice showed less activity at doses of 20 and 30 mg/kg of cocaine than periadolescent mice (*$P<0.05$) ($n=6-8$). Methylphenidate (10 mg/kg) induced less activity in postweanling than periadolescent and adult mice (*$P<0.05$).

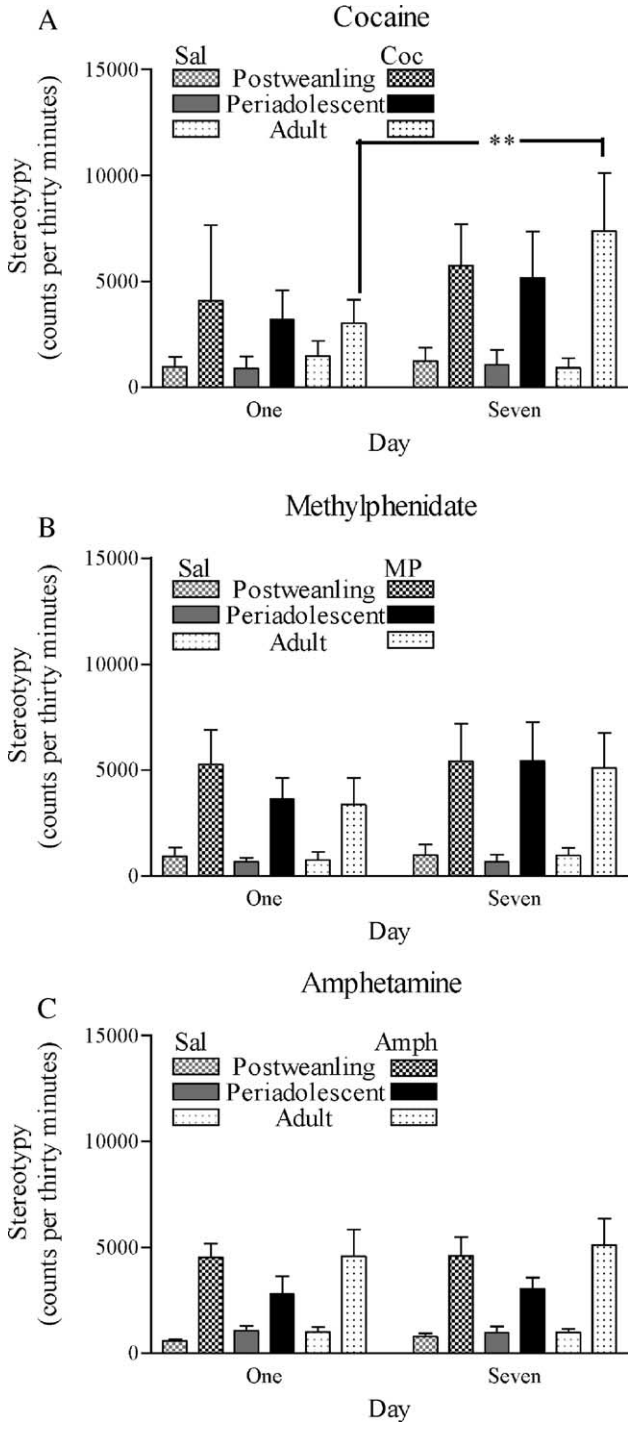

Fig. 4. Stereotypic counts in postweanling, periadolescent and adult mice following (A) cocaine (Coc) (20 mg/kg), (B) methylphenidate (MP) (10 mg/kg) or (C) amphetamine (Amph) (5 mg/kg) administration on days one and seven of the study. Stereotypic activity was increased following each psychostimulant injection. Adult mice showed higher stereotypic activity on day seven compared to day one following cocaine (**$P<0.01$), indicative of sensitization (A). Neither methylphenidate (B) nor amphetamine (C) produced behavioral sensitization in any age group ($n=6–16$).

seven in all of the groups. However, none of the groups had increased ambulatory activity when comparing day 1 to day 7, indicating no sensitization to the locomotor-activating properties of these drugs.

3.6. Repeated drug administration: total activity

Cocaine (20 mg/kg), methylphenidate (10 mg/kg), amphetamine (5 mg/kg) or saline (3 ml/kg) was administered once daily for seven days to postweanling, periadolescent and adult mice. Fig. 6 shows total activity on days one and seven of the

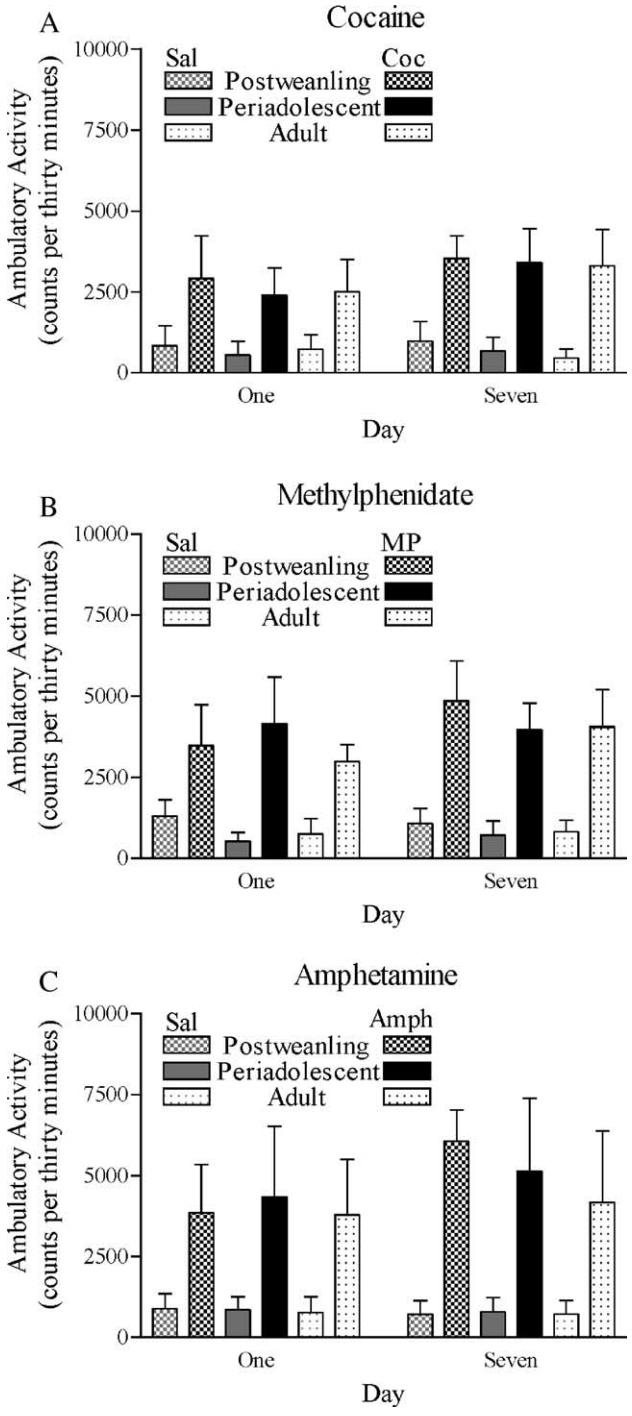

Fig. 5. Ambulatory activity in postweanling, periadolescent and adult mice following (A) cocaine (Coc) (20 mg/kg), (B) methylphenidate (MP) (10 mg/kg) or (C) amphetamine (Amph) (5 mg/kg) administration on days one and seven of the study. Ambulatory activity was increased following each psychostimulant injection. ($n=6–16$). Cocaine, methylphenidate and amphetamine did not produce behavioral sensitization in any age group.

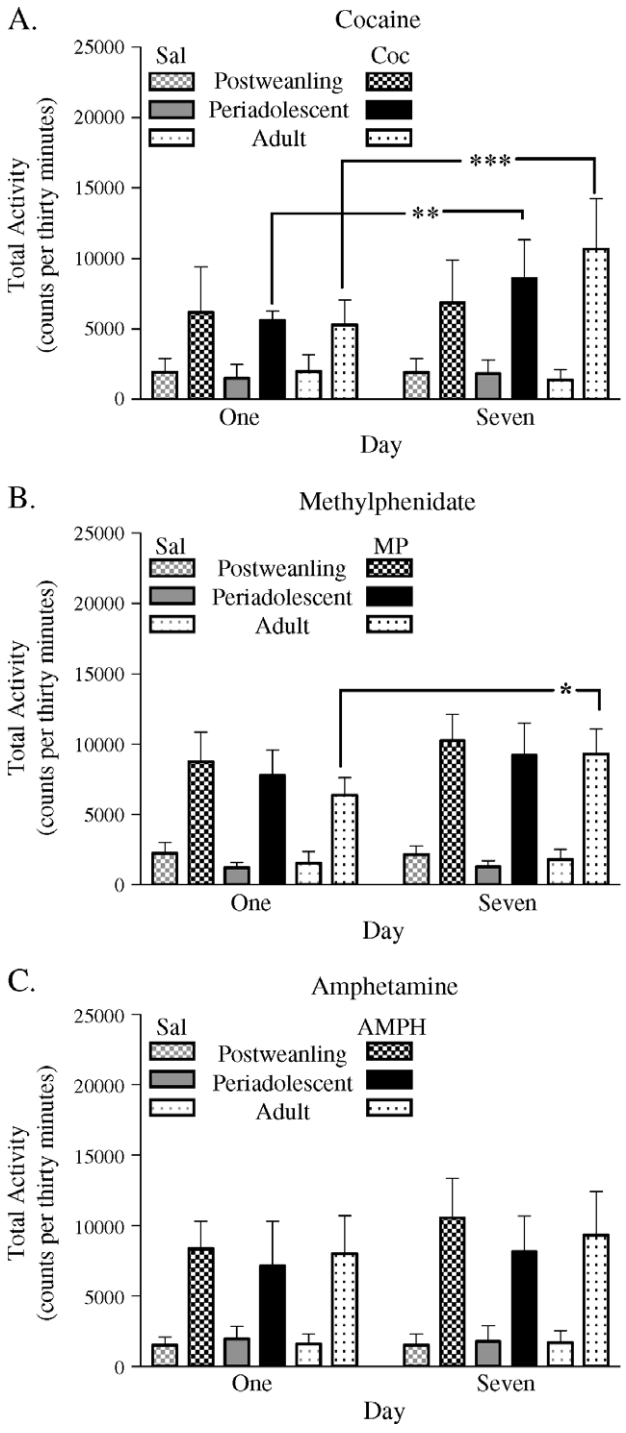

Fig. 6. Total activity in postweanling, periadolescent and adult mice following (A) cocaine (Coc) (20 mg/kg), (B) methylphenidate (MP) (10 mg/kg) or (C) amphetamine (Amph) (5 mg/kg) administration on days one and seven of the study. Total activity was increased following each psychostimulant injection. Periadolescent and adult mice showed higher activity on day seven compared to day one following cocaine (**$P<0.01$ and ***$P<0.001$, respectively), indicative of behavioral sensitization (A). Adult mice showed higher activity following methylphenidate administration on day seven compared to day one (*$P<0.05$) (B). Amphetamine did not produce behavioral sensitization in any age group (C) ($n=6–16$).

study. On both test days in all of the age groups, the activity of the cocaine-injected animals was significantly greater than the activity of those injected with saline. In both the periadolescent and adult mice, total activity following cocaine administration was significantly higher as determined by one-way ANOVA on day seven compared to day one [$F(3,28)=36.58$, $P<0.01$ and $F(3,25)=27.30$, $P<0.001$, respectively). In postweanling mice, there was no difference in activity on days one and seven (Fig. 6A), indicating a lack of sensitization to repeated cocaine. Methylphenidate (10 mg/kg) increased activity in postweanling, periadolescent and adult mice on days one and seven compared to saline-injected mice. Only adult mice had higher activity following methylphenidate on day seven than on day one as determined by one-way ANOVA followed by a Bonferonni post hoc [$F(5,47)=3.090$, $P<0.05$] (Fig. 6B). Amphetamine-induced activity was higher on days one and seven compared to saline in all age groups, but sensitization did not occur in any of the three age groups at this dose (Fig. 6C).

4. Discussion

Over the past decade, the initiation of psychostimulant exposure has occurred at an earlier age due to both the treatment of ADHD and illicit experimentation at younger ages. A rise in the reported incidence of ADHD resulted in a two- to three-fold increase in prescriptions for psychostimulants written for preschoolers between 1991 and 1995 (Zito et al., 2000). Also, according to The Monitoring the Future (MTF) survey in 1999, cocaine use in eighth-graders has doubled in comparison to those surveyed in 1991. It is well established that repeated psychostimulant exposure in adults can lead to long-lasting behavioral changes (reviewed by Nestler, 2004) and introducing these agents at younger ages even for medical reasons could adversely affect these individuals into adulthood. For instance, age of drug abuse onset has been shown to be inversely proportional to the extent of abuse that results (Kandel and Yamaguchi, 1993). In addition, children and adolescents are still undergoing developmental changes that could result in unique responses to psychostimulants as compared to adults.

In the present study, postweanling mice showed lower total, locomotor and stereotypic activity relative to periadolescent and adult mice following acute administration of the higher doses of methylphenidate, cocaine and amphetamine. Adult mice showed behavioral sensitization to both repeated methylphenidate and cocaine administration, whereas periadolescent mice were sensitized only to the motor-stimulating effects of cocaine, and to a lesser extent than the adults. Postweanling mice did not sensitize to the motor-activating or stereotypic properties of cocaine, methylphenidate or amphetamine.

The recent accumulation of data comparing the effects of psychostimulants in different aged rodents has been variable probably due to differences in drug dose and administration paradigm (Post et al., 1981) or species and strain of animal studied. Initially, it was reported that periadolescent rats are hyperactive at baseline (Spear and Brake, 1983) and that both periadolescent rats and mice are hypo-responsive to indirect

dopamine agonists such as amphetamine and cocaine when compared to younger and older animals (Spear and Brake, 1983; Laviola et al., 1999). However, groups have since not only shown lower baseline activity in periadolescent mice (Schramm-Saptya et al., 2004), but also a heightened sensitivity to amphetamine-induced behaviors, including reduced "lying still time", as well as increased "head-bobbing" in periadolescent rats (Laviola et al., 2001). Reports regarding sensitization to the behavioral activating properties of psychostimulants in different aged rats and mice have also been inconsistent (Laviola et al., 1995; Adriani et al., 1998; Collins and Izenwasser, 2002; Tirelli et al., 2003; Schramm-Saptya et al., 2004). Different procedures make comparisons between ages difficult; hence, a systematic approach to studying age-specific effects of drugs is warranted.

Age-specific behavioral responses following repeated psychostimulant exposure may implicate age-dependent sensitivities to the abuse potential of these drugs. Cocaine, methylphenidate and amphetamine increase extracellular synaptic dopamine concentrations in specific brain regions, including the caudate putamen and nucleus accumbens, which are associated with drug-induced activity and reinforcement (Kuczenski and Segal, 1997; Swerdlow et al., 1986; Volkow et al., 2001; Parsons and Justice, 1993; Everitt and Wolf, 2002). Repeated administration of these agents can result in a hypersensitization of the dopaminergic systems (Robinson and Berridge, 1993). A measure used in animals to test for this phenomenon is behavioral sensitization, a term which refers to the increased responsiveness to a given drug following prior exposure to the same drug (Post and Rose, 1976; Stewart and Badiani, 1993). Drug-induced sensitization of the dopaminergic system has been linked to certain aspects of the human state of addiction, including drug craving and drug seeking (Robinson and Berridge, 1993; Kalivas et al., 1998). Therefore, the results of the present study demonstrating the differential development of behavioral sensitization in mice during different stages of postnatal ontogeny suggest that age can play an important role in determining the potential for addiction.

Developing rodents are undergoing great changes involving the peak and subsequent pruning of many dopaminergic mediators including those associated with behavioral sensitization (Andersen and Teicher, 2000; Spear, 2000). While baseline D1 and D2 dopamine receptor levels peak in the striatum from 25 to 40 days old in rat (Teicher et al., 1995; Tarazi et al., 1999), dopamine transporter levels remain constant from 24 days old until adulthood in CD-1 mice (Ehrlich et al., 2002). Further, in the presence of psychostimulants, previous evidence suggests that developing rodents can be either hypo- or hyper-responsive, exhibiting increased or decreased neuronal plasticity in the brain systems associated with psychomotor activity (Fukui et al., 2003; Andersen, 2002; Adriani and Laviola, 2004; Leff et al., 1984). According to the hypo-responsive hypothesis, psychostimulant-induced changes in the neurochemical pathways thought to be involved in behavioral sensitization are not yet developed, and therefore muted. Fukui et al. showed that cocaine- and methylphenidate-stimulated responses via D1 dopamine receptor signaling are lower in young (21–22 day old) mice compared to adult (6–8 week old mice) mice (Fukui et al., 2003). Previous rodent studies have also shown reduced responsiveness to psychostimulants during hyperdopaminergic states, i.e. puberty (Andersen, 2002). It is also possible that the converse is occurring, and an increased plasticity in the developing brain allows for the establishment of homeostasis more readily. For example, nicotine down-regulates striatal levels of the α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) GluR2/3 subunit in periadolescent rodents, while eliciting no effects on adult levels, indicating heightened neuroplasticity in younger animals (Adriani and Laviola, 2004). Also, young rats (22–30 days old) have increased regeneration of D2 dopamine receptors when compared to adult mid-life rats (9–12 months old) following administration of an irreversible dopamine receptor antagonist (Leff et al., 1984). In addition, there is a peak during the postweanling period of brain-derived neurotrophic factor (BDNF) and the receptor which it activates, TRK_B, which are associated with neural growth that could account for increased plasticity during this period (Yurek et al., 1998; Ringstedt et al., 1993). According to this postulation, postweanling mice are undergoing increased neural plasticity, re-establishing homeostasis, and therefore blocking psychostimulant-induced sensitization.

Interestingly, in the present study there were no differences between periadolescent and adult mice, either in baseline or acute drug-induced activity, contrary to some previous reports (Cirulli et al., 1996; Laviola et al., 1999; Adriani and Laviola, 2000; Schramm-Saptya et al., 2004; Vila et al., 2004). Adriani and Laviola report that distinct behavioral profiles between ages can be abolished if a habituation period is included in the testing procedure (Adriani and Laviola, 2000). Therefore it is possible that the lack of unique baseline behaviors within the postnatal developmental stages described in our study was due to the 30 min acclimation period we employed in our experimental paradigm.

One possible explanation for age-dependent responses to psychostimulants lies in differences in drug metabolism. Comparisons between periadolescent and adult male CD-1 mice demonstrate that the younger mice have lower brain levels of both cocaine and amphetamine following an acute injection suggesting more rapid drug metabolism in the periadolescent mice. The differences in brain drug levels, however did not correlate with behavior in that study (McCarthy et al., 2004). Likewise, in the present study, periadolescent and adult mice showed similar behavioral responses to acute cocaine and amphetamine despite presumably lower drug brain concentrations in the adolescents. This suggests that the age-specific responses to cocaine and amphetamine are not always explained by differences in drug metabolism. However, further studies of the pharmacokinetics of psychostimulants in postweanling mice are needed to draw definitive conclusions regarding the role of kinetics in age-related behavioral effects of these drugs.

Within our consistent sensitization paradigm, postweanling mice were not sensitized to the activity-inducing properties of

cocaine, methylphenidate or amphetamine. Periadolescent mice were only sensitized to cocaine-induced increases in activity, whereas adult mice exhibited behavioral sensitization to cocaine and methylphenidate, but not amphetamine. In general, these data imply that the acquisition of sensitization increases with age. Within the confines of both the hypo- and hyper-responsiveness hypotheses, the absence of behavioral sensitization, thought to be related to a hypersensitization of the systems that mediate certain behaviors linked to the addictive state in humans, suggests that an immature nervous system may have a lower altered susceptibility to drug abuse. Cocaine also has been shown to be less reinforcing in 31-day-old rats as compared to older rats (41- and 95-day-old) using a self-administration procedure (Leslie et al., 2004), furthering the hypothesis that the developing brain responds differentially to the effects of psychostimulants.

The present study showed that adult animals were sensitized to the behavioral activating effects of repeated, intermittent administration of methylphenidate, which is congruent with some of the reported literature (Gaytan et al., 1997; Crawford et al., 1998; Shuster et al., 1982). However, others show tolerance to repeated methylphenidate (McNamara et al., 1993; Crawford et al., 1998) and still another study showed neither tolerance nor sensitization following either continuous infusion or repeated injections of methylphenidate in the rat (Izenwasser et al., 1999). Within our systematic study adult mice, but not periadolescent or postweanling mice, were sensitized to the increases in activity induced by methylphenidate furthering the theory that the acquisition of behavioral sensitization increases with age.

While adults showed behavioral sensitization to methylphenidate and both adults and periadolescents were sensitized to cocaine, none of the age groups sensitized to the locomotor or stereotypic activating properties of amphetamine. This is an interesting finding in that all of these drugs are indirect dopamine agonists. However, while cocaine and methylphenidate are both reuptake inhibitors, amphetamine promotes neurotransmitter release. These drugs also differ somewhat in their actions on serotonin and norepinephrine. Perhaps the difference in mechanism of action accounts for the different response seen following repeated administration of amphetamine compared to methylphenidate or cocaine. Adriani et al. have shown that both adults and periadolescent CD-1 mice sensitize to the locomotor-stimulating effects of 2 mg/kg amphetamine, using a procedure that includes a 48-h withdrawal period (Adriani et al., 1998). We used a larger dose of amphetamine (5 mg/kg) which could be responsible for the different outcomes. We also did not have a withdrawal period between the repeated drug administration procedure and the test for sensitization, which could influence the results towards less expression of sensitization. Other studies have shown that sensitization is increased with longer withdrawal periods between the pretreatment and the drug challenge and test for expression of sensitization (DiCiano et al., 2002; Todtenkopf et al., 2002).

In summary, generalizations regarding age-specific responses to psychostimulants are difficult to make in the presence of differing paradigms producing conflicting results. However, because the present results were obtained from the identical administration pattern of three psychostimulants to different aged mice, it can be said that postweanling mice differ in their behavioral responses to psychostimulants relative to periadolescent and adult mice. The direction of the difference depends on the measurement, drug and dose. These data may have implications for the abuse liability of psychostimulants during different stages of development.

References

Adriani W, Laviola G. A unique hormonal and behavioral hyporesponsivity to both forced novelty and D-amphetamine in periadolescent mice. Neuropharmacology 2000;39(2):334–46.

Adriani W, Laviola G. Windows of vulnerability to psychopathology and therapeutic strategy in the adolescent rodent model. Behav Pharmacol 2004;15(5 and 6):341–52.

Adriani W, Chiarotti F, Laviola G. Elevated novelty seeking and peculiar D-amphetamine sensitization in periadolescent mice compared with adult mice. Behav Neurosci 1998a;112(5):1152–66.

Andersen SL. Changes in the second messenger cyclic AMP during development may underlie motoric symptoms in attention deficit/hyperactivity disorder (ADHD). Behav Brain Res 2002;130(1-2):197–201.

Andersen SL, Teicher MH. Sex differences in dopamine receptors and their relevance to ADHD. Neurosci Biobehav Rev 2000;24(1):137–41.

Cantwell DP. Attention deficit disorder: a review of the past 10 years. J Am Acad Child Adolesc Psychiatry 1996;35(8):978–87.

Cirulli F, Laviola G. Paradoxical effects of D-amphetamine in infant and adolescent mice: role of gender and environmental risk factors. Neurosci Biobehav Rev 2000;24(1):73–84.

Cirulli F, Terranova ML, Laviola G. Affiliation in periadolescent rats: behavioral and corticosterone response to social reunion with familiar or unfamiliar partners. Pharmacol Biochem Behav 1996;54(1):99–105.

Collins SL, Izenwasser S. Cocaine differentially regulates both behavior and neurochemistry in periadolescent versus adult rats. Brain Res Dev Brain Res 2002;138(1):27–34.

Connor CE, Kuczenski R. Evidence that amphetamine and Na+ gradient reversal increase striatal synaptosomal dopamine synthesis through carrier-mediated efflux of dopamine. Biochem Pharmacol 1986;35(18):3123–30.

Crawford CA, McDougall SA, Meier TL, Collins RL, Watson JB. Repeated methylphenidate treatment induces behavioral sensitization and decreases protein kinase A and dopamine-stimulated adenylyl cyclase activity in the dorsal striatum. Psychopharmacology (Berl) 1998;136(1):34–43.

DiChiara G. The role of dopamine in drug abuse viewed from the perspective of its role in motivation. Drug Alcohol Depend 1995;38:95–137.

DiCiano P, Everitt BJ. Reinstatement and spontaneous recovery of cocaine-seeking following extinction and different durations of withdrawal. Behav Pharmacol 2002;13:397–405.

Ehrlich ME, Sommer J, Canas E, Unterwald EM. Periadolescent mice show enhanced ΔFosB upregulation in response to cocaine and amphetamine. J Neurosci 2002;22:9155–9.

Everitt BJ, Wolf ME. Psychomotor stimulant addiction: a neural systems perspective. J Neurosci 2002;22:3312–20.

Fukui R, Svenningsson P, Matsuishi T, Higashi H, Nairn AC, Greengard P, et al. Effect of methylphenidate on dopamine/DARPP signaling in adult, but not young, mice. J Neurochem 2003;87:1391–401.

Gaytan O, al-Rahim S, Swann A, Dafny N. Sensitization to locomotor effects of methylphenidate in the rat. Life Sci 1997;61(8):L101–7.

Gerasimov MR, Franceschi M, Volkow ND, Gifford A, Gatley SJ, Marsteller D, Molina PE, Dewey SL. Comparison between intraperitoneal and oral methylphenidate administration: a microdialysis and locomotor activity study. J Pharmacol Exp Ther 2000;295(1):51–7.

Heffner TG, Seiden LS. Possible involvement of serotonergic neurons in the reduction of locomotor hyperactivity caused by amphetamine in neonatal rats depleted of brain dopamine. Brain Res 1982;244(1):81–90.

Heikkila RE, Orlansky H, Cohen G. Studies on the distinction between uptake inhibition and release of [^{3}H] dopamine in rat brain tissue slices. Biochem Pharmacol 1975a;24(8):847–52.

Heikkila RE, Orlansky H, Mytilineou C, Cohen G. Amphetamine: evaluation of D- and L-isomers as releasing agent and uptake inhibitors for 3H-dopamine and 3H-norepinephrine in slices of rat neostriatum and cerebral cortex. J Pharmacol Exp Ther 1975b;194(10):47–56.

Hummel M, Ansonoff MA, Pintar JE, Unterwald EM. Genetic and pharmacological manipulation of mu opioid receptors in mice reveals a differential effect on behavioral sensitization to cocaine. Neuroscience 2004;125(1): 211–20.

Izenwasser S, Coy AE, Ladenheim B, Loeloff RJ, Cadet JL, French D. Chronic methylphenidate alters locomotor activity and dopamine transporters differently from cocaine. Eur J Pharmacol 1999;373(2–3):187–93.

Kalivas PW, Pierce RC, Cornish J, Sorg BA. A role for sensitization in craving and relapse in cocaine addiction. Psychopharmacology 1998;12(1):49–53.

Kandel D, Yamaguchi K. From beer to crack: developmental patterns of drug involvement. Am J Public Health 1993;83(6):851–5.

Koe BK. Molecular geometry of inhibitors of the uptake of catecholamines and serotonin in synaptosomal preparations of rat brain. J Pharmacol Exp Ther 1976;199:649–61.

Kuczenski R, Segal DS. Effects of methylphenidate on extracellular dopamine, serotonin and norepinephrine: comparisons with amphetamine. J Neurochem 1997;68:2032–7.

Kuczenski R, Segal DS, Todd PK. Behavioral sensitization and extracellular dopamine responses to amphetamine after various treatments. Psychopharmacology 1997;134:221–9.

Laviola G, Wood RD, Kuhn C, Francis R, Spear LP. Cocaine sensitization in periadolescent and adult rats. J Pharmacol Exp Ther 1995; 275(1):345–57.

Laviola G, Adriani W, Terranova ML, Gerra G. Psychobiological risk factors for vulnerability to psychostimulants in human adolescent and adult animals. Neurosci Biobehav Rev 1999;23:993–1010.

Laviola G, Pascucci T, Pieretti S. Striatal dopamine sensitization to D-amphetamine in periadolescent but not in adult rats. Pharm Biochem Behav 2001;68:115–24.

Leff SE, Gariano R, Creese. Dopamine receptor turnover rates in rat striatum are age-dependent. Proc Natl Acad Sci 1984;81:3910–4.

Leslie FM, Loughlin SE, Wang R, Perez L, Lotfipour S, Belluzzi JD. Adolescent development of forebrain stimulant responsiveness: insights from animal studies. Ann N Y Acad Sci 2004;1021:148–59.

McCarthy LE, Mannelli P, Niculescu M, Gingrich K, Unterwald EM, Ehrlich ME. The distribution of cocaine in mice differs by age and strain. Neurotoxicol Teratol 2004;26(6):839–48.

McDougall SA, Collins RL, Karper PE, Watson JB, Crawford CA. Effects of repeated methylphenidate treatment in the young rat: sensitization of both locomotor activity and stereotyped sniffing. Eur J Pharmacol 1999;373(2-3):187–93 Jun 4.

McNamara CG, Davidson ES, Schenk S. A comparison of the motor-activating effects of acute and chronic exposure to amphetamine and methylphenidate. Pharmacol Biochem Behav 1993;45(3):729–32.

Nestler EJ. Historical review: molecular and cellular mechanisms of opiate and cocaine addiction. TIPS 2004;25(4):210–8.

Parsons LH, Justice Jr JB. Serotonin and dopamine sensitization in the nucleus accumbens, ventral tegmental area, and dorsal raphe nucleus following repeated cocaine administration. J Neurochem 1993;61:1611–9.

Post RM, Rose H. Increasing effects of repetitive cocaine administration in the rat. Nature (London) 1976;260:731–2.

Post RM, Lockfield A, Squillace KM, Contel NR. Drug-induced environment interaction: context dependency of cocaine-induced behavioral sensitization. Life Sci 1981;28:755–60.

Rapoport JL, Buchsbaum MS, Weingartner H, Zahn TP, Ludlow C, Mikkelsen EJ. Dextroamphetamine. Its cognitive and behavioral effects in normal and hyperactive boys and normal men. Arch Gen Psychiatry 1980;37:933–43.

Ringstedt T, Lagercrantz H, Persson H. Expression of members of the trk family in the developing postnatal rat brain. Brain Res Dev Brain Res 1993;72(1):119–31.

Robinson TE, Berridge KC. The neural basis of drug craving: an incentive-sensitization theory of addiction. Brain Research Reviews 1993;18:247–91.

Schramm-Saptya NL, Pratt AR, Winder DG. Effects of periadolescent versus adult cocaine exposure on cocaine conditioned place preference and motor sensitization in mice. Psychopharmacology 2004;173:41–9.

Shuster L, Hudson J, Anton M, Righi D. Sensitization of mice to methylphenidate. Psychopharmacology (Berl) 1982;77(1):31–6.

Spear LP. The adolescent brain and age-related behavioral manifestations. Neurosci Biobehav Rev 2000;24:417–63.

Spear LP, Brake SC. Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. Dev Psychobiol 1983;16:83–109.

Stewart J, Badiani A. Tolerance and sensitization to the behavioral effects of drugs. Behav Pharmacol 1993;4:289–312.

Swerdlow NR, Vaccarino FJ, Amalric M, Koob GF. The neural substrates for the motor-activating properties of psychostimulants: a review of recent findings. Pharmacol Biochem Behav 1986;25(1):233–48.

Tarazi FI, Tomasini EC, Baldessarini RJ. Postnatal development of dopamine D1-like receptors in rat cortical and striatolimbic brain regions: an autoradiographic study. Dev Neurosci 1999;21(1):43–9.

Teicher MH, Andersen SL, Hostetter Jr JC. Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. Brain Res Dev Brain Res 1995;89(2):167–72.

Tirelli E, Laviola G, Adriani W. Ontogenesis of behavioral sensitization and conditioned place preference induced by psychostimulants in laboratory rodents. Neurosci Biobhav Rev 2003;27(1–2):163–78.

Todtenkopf MS, Mihalakopoulos A, Stellar JR. Withdrawal duration differentially affects c-*fos* expression in the medial prefrontal cortex and discrete subregions of the nucleus accumbens in cocaine-sensitized rats. Neurosci 2002;114(4):1061–9.

Tzschentke TM, Schmidt WJ. Glutamatergic mechanisms in addiction. Mol Psychiatry 2003;8:373–82.

Vanderschuren LJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology 2000;151:99–120.

Vila JL, Philpot RM, Kirstein CL. Grid crossing: inability to compare activity levels between adolescent and adult rats. Ann N Y Acad Sci 2004;1021: 418–21.

Volkow ND, Wang G, Fowler JS, Logan J, Gerasimov M, Maynard L. Therapeutic doses of oral methylphenidate significantly increase extracellular dopamine in the human brain. J Neurosci 2001;21:RC121.

Wolf ME. The role of excitatory amino acids in behavioral sensitization to psychomotor stimulants. Prog Neurobiol 1998;54:679–720.

Yurek DM, Hipkens SB, Wiegand SJ, Altar CA. Optimal effectiveness of BDNF for fetal nigral transplants coincides with the ontogenic appearance of BDNF in the striatum. J Neurosci 1998;18(15):6040–7.

Zito JM, Safer DJ, dosReis S, Gardner JF, Boles M, Lynch F. Trends in prescribing psychotropic medications to preschoolers. JAMA 2000;283: 1025–30.