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Striatal dopamine sensitization to D-amphetamine in periadolescent but not in adult rats

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Abstract

The neurobiological and behavioral facets of adolescence have been poorly investigated in relation to the vulnerability to psychostimulants. Periadolescent (33–43 days) and adult (>70 days) Sprague–Dawley rats underwent a 3-day treatment history with D-amphetamine (AMPH) at 0, 2, or 10 mg/kg (once a day). After a short 5-day-long withdrawal interval, freely moving animals were challenged with a 2-mg/kg AMPH dose and their behavior as well as in vivo intrastriatum dopamine (DA) release in the CNS were assessed. Microdialysis data indicated that AMPH-history periadolescent rats showed a prominent sensitization of AMPH-stimulated DA release, whereas no such change was found in adult subjects. As expected, acute AMPH administration strongly reduced time spent *lying still* and increased levels of cage *exploration* in animals of both ages. A treatment history of high AMPH dosage was associated with a marked sensitization of the exploratory behavior in adults, whereas it induced a quite opposite profile in periadolescents. The latter group only was also characterized by a compulsive involvement in the stereotyped *head-bobbing* response. These results indicate that differently from adults, marked alterations in neurobiological target mechanisms are observed in rats around periadolescence as a consequence of a quite mild regimen of intermittent AMPH exposure. Thus, a neurobiological substrate for an age-related increased vulnerability towards the addictive risks of these drugs is suggested. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Risk factors; Adolescence; Behavioral sensitization; Microdialysis; Dopamine system; D-Amphetamine; Rat

1. Introduction

Recent research has emphasized that an increased risk of developing drug-abuse and drug-related problems is associated with the adolescent period (Anthony and Petronis, 1995; Breslau and Peterson, 1996), during which different patterns of temporary deviance and the use of various kinds of psychoactive agents are quite often observed (Compas et al., 1995; Mathias, 1996; Newcomb, 1985; Wills et al., 1994). Adolescence is a unique ontogenetic period during which plasticity of the brain continues through neuroanatomical, neurochemical, and neurophysiological processes (Teicher et al., 1997). However, although it is during adolescence that most drug use and abuse patterns are initiated, there have been relatively few investigations of the factors contributing to this age-specific propensity (Estroff et al., 1989). Very little is also known about the unique effects and consequences that the exposure to potent psychoactive agents may have during this developmental period (for a review, see Laviola et al., 1999; Spear, 2000). In fact, the alterations that occur in the neurobiological target mechanisms may produce changes that make continued drug use more likely.

When compared with younger or older animals, periadolescent rats and mice show marked changes in several significant aspects of baseline behavior and physiology (Choi and Kellogg, 1996; Cirulli et al., 1996; Terranova et al., 1999), as well as alterations in psychopharmacological sensitivity (Bolanos et al., 1998; Laviola et al., 1999; Spear, 2000; Spear and Brake, 1983). In particular, animals of this age exhibit an attenuated behavioral response to acute administration of indirect dopamine (DA) agonists such as cocaine and D-amphetamine (AMPH), and an accentuated behavioral response to a catecholamine antagonist (Laviola et al., 1999; Shalaby et al., 1981; Spear and Brake, 1983).

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The general profile apparently does not rely on an age-specific decrease in brain-drug availability (Campbell et al., 1988), but rather appears to be related to an alteration in nervous system sensitivity.

Certain patterns of consumption, as well as the likelihood to shift from use to abuse and the chances of developing dependence both in animals and humans, all seem to be positively correlated with the development of both tolerance and sensitization to drug effects after repeated administration of the same agent (Goudie and Emmett-Oglesby, 1989) (for literature see McDougall et al., 1994). In animal models, repeated and intermittent administration of psychostimulants such as cocaine or AMPH results in a progressive augmentation in spontaneous locomotor activity or in a stereotyped behavioral syndrome (Koff et al., 1994; Laviola et al., 1988, 1992, 1994; Post and Rose, 1976; Segal, 1986). Such behaviors are thought to be mediated by the mesolimbic and nigrostriatal DA pathways, respectively (Fontana et al., 1993; Goudie and Emmett-Oglesby, 1989; Robinson and Berridge, 1993; Staton and Solomon, 1994).

Sensitization phenomena at developmental ages have been poorly investigated, yet important ontogenetic changes in the neurobiological systems underlying the development of sensitization may be expected, and this in turn might be responsible for different levels of vulnerability to drugs at different developmental ages (Kolta et al., 1990; Laviola et al., 1995, 1999; McDougall et al., 1994; Wood et al., 1998). In this framework, a series of very recent studies by our group compared the development of AMPH-induced sensitization in adult mice and in subjects observed during periadolescence (Adriani et al., 1998; Adriani and Laviola, 1999). The results evidenced prominent and peculiar age-related differences in the behaviors elicited in response to the same regimen and range of drug doses administered (see also Laviola et al., 1994, 1995). To further address this issue, it seemed important to carry out a deeper and concomitant analysis in the same animal, on the development of both behavioral and neurochemical sensitization to chronic and intermittent AMPH administration on animals of the two ages (namely adults and periadolescents).

In contrast to the behavioral sensitization induced by chronic exposure to DA antagonists (Antellman et al., 1986; Vanzina and Stewart, 1989), the neural mechanisms responsible for sensitization following repeated agonist treatments are still not well understood (Kalivas et al., 1993). Thus, an increasing interest has emerged in characterizing the nature of persistent drug-induced adaptations in the nervous system responsible for these behavioral profiles. There is evidence that pre- as well as postsynaptic alterations in dopaminergic neurotrasmission are relevant for the manifestation of the sensitization phenomena (Stewart and Badiani, 1993). Some investigators, by using in vivo brain microdialysis, have reported that behavioral sensitization to elevated dosages of AMPH is accompanied by an increase in DA release in the caudate–putamen and in the nucleus accumbens (Lienau and Kuschinsky, 1997; Patrick et al., 1991; Paulson and Robinson, 1995; Robinson et al., 1988). A sensitization-related enhancement in AMPH-stimulated DA release using tissue from these two brain areas was also observed (Yamada et al., 1998). Other authors, however, reported that behavioral sensitization to AMPH-like drugs can be obtained in the absence of an enhancement in DA release in response to the drug challenge (Kolta et al., 1985; Segal and Kuczenski, 1987; Wolf et al., 1992).

In keeping with these considerations, it seemed important to conduct a concomitant observational behavioral as well as a time-course microdialysis investigation on the same animal. This included an investigation on possible age-related differences in CNS dopaminergic system function as a consequence of repeated and intermittent treatment with AMPH (Tsuchida et al., 1994). Since increased motor behaviors are thought to rely on AMPH-induced stimulation of the DA pathways in the caudate–putamen area in the CNS (Kelly et al., 1975; Staton and Solomon, 1994), the release of DA and its metabolites in response to an AMPH challenge was investigated in the striatum area of the CNS in freely moving rats of the two ages by means of in vivo microdialysis.

2. Methods

2.1. Subjects

Sprague–Dawley pregnant rats were obtained from Charles River Italia (Calco, Italy). Upon arrival, animals were maintained in an air-conditioned room at $21 \pm 1^{\circ}C$ and $50 \pm 10\%$ relative humidity and housed separately in $40 \times 25 \times 20$-cm Plexiglas boxes, with sawdust as bedding and with a metal top. The animals were housed in a 12:12 h light/dark cycle with lights on at 9:30 a.m. Food (enriched standard diet purchased from Piccioni, Brescia, Italy) and water were freely available.

Females were housed individually and inspected daily for delivery. The following day, litters were culled to six males. On postnatal day (pnd) 21, rat pups were weaned, with three littermates being housed in each Plexiglas cage ($33 \times 13 \times 14$). Rats were randomly assigned for testing at one of two different postnatal ages, namely, periadolescence (pnd 33–43) or adulthood (pnd 61–71). Each subject was tested individually at one age only.

2.2. General procedure

The whole experimental schedule took a total of 9 days, subjects from both age groups being tested between 10:00 a.m. and 5:00 p.m. Testing of different experimental group was counterbalanced across time.

2.2.1. Days 1, 2, 3: pretreatment period

On each of these 3 days, periadolescent and adult rats were weighed and administered an AMPH injection (0, 2, or

10 mg/kg, ip). Immediately after the injection, animals were returned to their home cage

2.2.2. Days 4 to 8 (wash-out)

A wash-out interval of 5 days was left between the last day of pretreatment and the challenge day, in order to avoid the influence of residual circulating levels of AMPH during the testing session. In addition, sensitized behavioral responses are widely reported using this time interval (for the role of withdrawal interval in the development of behavioral and neurochemical sensitization, see Paulson and Robinson, 1995)).

2.2.3. Challenge day (concomitant behavioral and microdialysis investigation in the same animal)

Five days after the last drug injection, all animals received an intraperitoneal injection of a standard AMPH (2 mg/kg) dose in the test chamber to determine the effects of the different treatment procedures (treatment history) on responsiveness to challenge with the same drug upon novelty exposure.

The doses and route of administration were based on previous studies evaluating the influence of repeated amphetamine exposure on the behavioral and neuroendocrine responses (Laviola and Adriani, 1998; Laviola et al., 1999). Body weight gain was recorded daily for each animal to monitor the effects of chronic AMPH exposure on this measure.

2.3. Apparatus

The testing chambers consisted of transparent Plexiglas bowls (40-cm high), which were located in a room separated from the colony room and kept in standard conditions.

2.4. Microdialysis technique

2.4.1. Surgery

Twenty-four hours before the test (challenge) day, rats were anesthetized with Equitesin (3 ml/kg dosage, ip) (chloral hydrate 2 g, pentobarbital sodium 450 mg, $MgSO_4$ 1 g, propylene glycol 17 ml, ethyl alcohol 95° 7 ml, distilled water 26 ml) and placed in a stereotaxic frame (David Kopf Instruments Mod. 900, Elmo St., Tujunga, CA, USA). Body temperature was kept stable using infrared light. The skull was exposed and a hole was drilled for implantation of a microdialysis probe (CMA 12 microdialysis probe, 3-mm length, 0.5-mm od, Carneige Medicin, Solna, Sweden) whose tip was located in the striatum (young rat coordinates: AP +1.4; ML +2.1; DV −5; adult rat coordinates: AP +1.7; ML +2.8; DV −7, according to Paxinos and Watson (1996). Skull screws and dental cement were used to anchor the probe to the skull. At the end of surgery, animals were allowed to recover from anaesthesia and were placed in a plastic bowl with free access to food and water. The location of dialysis probe was verified histologically by sectioning the brain in a cryostat and staining with cresyl violet.

2.5. Microdialysis sampling

Microdialysis probe was connected via FEP-tubing and a dual-channel swivel device to the syringe of a CMA/100 microinjection pump (Carneige Medicin) and perfused with degassed and filter-sterilized Ringer solution (Na^+ 147 mM, K^+ 4 mM, Ca^{2+} 2.3 mM, Cl^- 156 mM, pH 6.0) at a flow rate of 0.2 ml/min. After 24 h, the flow rate was adjusted at 2 ml/min and dialysate samples were collected at 20-min intervals in polyethylene tubes containing 10 ml of 0.1 M $HClO_4$ and placed in a refrigerated collector (CMA/140, Carneige Medicin). Animals were followed for a 3-h equilibration period and four samples, where amine values did not change more than 10%, were collected to determine basal levels. Then amphetamine challenge was administered intraperitoneally and microdialysis performed for another 2 h. At the end of the dialysis session, samples were stored at −30°C for later HPLC analysis.

2.6. Sample HPLC analysis

Dialysates were injected without any further preparation in a volume of 40 ml by a CMA/200 refrigerated microsampler (Carneige Medicin) into a reversed-phase chromatographic apparatus (Gilson 506 pump and Gilson 805 manometric module mastered by a Gilson Unipoint system software, Gilson Italia, Milan, Italy) 2 to 3 days after microdialysis sessions. Isocratic separation was achieved at ambient temperature using a Supelco LC-18 DB column (od 5 mm, length 15 cm, and id 4.6 mm, Supelco, Bellefonte, PA, USA). The mobile phase consisted of acetate buffer (12 mM sodium acetate, 0.26 mM Na_2EDTA, 0.5 mM octane sulphonic acid sodium salt, pH 2.6)–methanol (86:14, v/v). The mobile phase was filtered and degassed. The flow rate of the mobile phase was 1.0 ml/min and one complete sample run was less than 20 min. DA and metabolites were detected by a glassy carbon amperometric electrode set at +0.75 V (Gilson 142, Gilson Italia) vs. an Ag/AgCl reference electrode. The detection limit of the assay was 0.03 pmol/sample.

2.7. Behavioral analysis

A time-course behavioral analysis was performed during the light phase of the light/dark cycle on the challenge day. On this day, each rat implanted with the microdialysis probe was gently placed in the test chamber to which it was unfamiliar and videorecorded for a total of six 5-min samples across the 30-min observation session immediately postinjection. The behavioral profile expressed by each animal was subsequently scored by means of an IBM computer and specific software (THE OBSERVER v2.0 for DOS, Noldus Information Technology, Wageningen, the

Netherlands). This allowed a detailed analysis of several parameters, such as frequency and duration of each behavior. The behaviors measured included *lying still* (absence of any gross movement), *exploration* of the cage environment and *head-bobbing* (stereotyped, repetitive movements of the head, either up-and-down and directed toward one wall of the chamber, or side-to-side motions) (Laviola et al., 1995). Behavioral scoring was conducted by trained experimenters blind to the treatment condition of the animals.

2.8. Drugs

AMPH was dissolved in SAL (NaCl, 0.9%) and injected intraperitoneally in a volume of 1 ml/kg body weight. AMPH doses have been chosen in the range of those used in previous studies (Laviola and Adriani, 1998), in order to maximize the observation of several kinds of behavioral responses, including stereotypy.

2.9. Design and data analysis

The design of the experiment was a 2 Age (periadolescent vs. adult) × 3 Treatment history (0, 2, or 10 mg/kg AMPH) combination, with only one pup per litter being randomly placed into each of the experimental groups at a given age. Data from each behavioral measure were analyzed separately by analysis of variance (ANOVA) with a repeated within-subject factor. When appropriate, post hoc comparisons were performed using the Tukey HSD test to determine the locus of significant effects.

All experimental procedures have been performed in accordance with the guidelines of the European Community Directive of 24 November 1986 (86/609/EEC).

3. Results

3.1. Response to an AMPH challenge (testing day)

3.1.1. Brain microdialysis

As a whole, basal levels of DA and its metabolites DOPAC and HVA in the striatum area showed a significant difference as a function of the age of the animals, $F(1,36) = 62.604$, 41.129, 82.51, P's $< .001$, respectively, with periadolescents' values being consistently lower than those of adults (Table 1).

With respect to the consequences of repeated and intermittent drug administration, the ANOVA revealed a significant interaction of age by treatment history for DA levels, $F(2,36) = 3.107$, $P < .05$. As shown in Fig. 1 (upper panels), the expected increment in DA levels in response to an acute AMPH challenge (see empty symbols, subjects receiving the drug for the first time) was significantly more marked in adult than in periadolescent SAL-history rats ($P < .05$ or less for the 2nd, 3rd, and 6th interval). This finding confirms recent endocrine as well as behavioral literature showing hyposensitivity to acute psychostimulant effects in the latter age group.

Table 1
Age-related changes in basal extracellular levels (picomoles per sample) of DA, DOPAC, and HVA (mean ± S.D.)

Age	DA	DOPAC	HVA
Periadolescents	0.06 (±0.03)	24.75 (±9.3)	17.35 (±5.9)
Adults	0.15 (±0.04)[a]	41.32 (±2.8)[a]	32.82 (±3.3)[a]

[a] Significantly different from periadolescent groups.

When looking at the carryover effects of a treatment history with the same drug (closed symbols), in the absence of changes in the adult group, a prominent sensitization was found in AMPH-history periadolescents. In fact, these animals exhibited much higher DA concentrations when compared to the corresponding SAL treatment-history group (for post hoc results, see legend of Fig. 1).

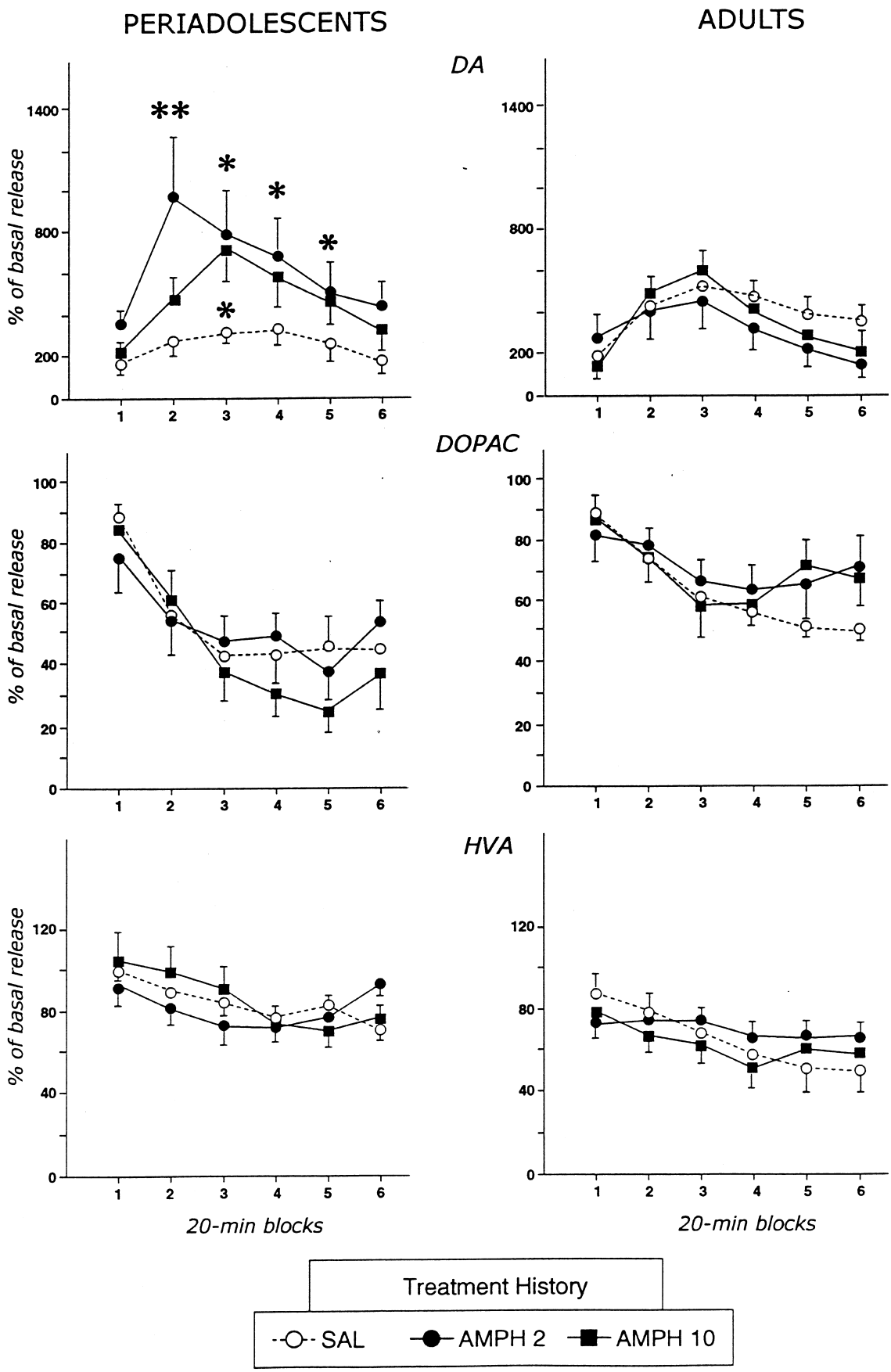
For DOPAC levels (middle panels), significant main effects of age appeared, $F(1,33) = 12.43$, $P < .001$, with the periadolescent group values being as a whole approximately 15% lower than adults. As a whole, DOPAC levels showed a decreasing profile over the 120-min session after the AMPH injection, $F(5,180) = 37.645$, $P < .001$ and no significant changes were found in response to AMPH or as a function of each animal's treatment history with the same drug.

For HVA levels (low panels), a decreasing profile was found over the 120-min session, $F(5,180) = 16.495$, $P < .001$, and again a significant age difference appeared, $F(1,36) = 12.116$, $P < .01$. Periadolescents showed as a whole almost a 10% higher levels than adults. Furthermore, the ANOVA yielded a significant interaction of age by treatment history over the session, $F(10,180) = 2.310$, $P < .01$. A mixed profile was found, with periadolescents from the high-AMPH-history group showing, in response to an acute drug challenge, a slower reduction over the session than the corresponding adult group.

3.2. Behavioral analysis

With the aim of assessing the development of carryover effects of repeated and intermittent AMPH administration, an ANOVA was performed on data comparing SAL animals with those that received 2 or 10 mg/kg AMPH chronically. In these analyses, main effects of age were seen, with adults

Fig. 1. Mean (±S.E.M.) DA, DOPAC, and HVA levels collected in the striatum area of periadolescent and adult rats in response to a challenge with a standard AMPH (2 mg/kg) dose in a novel environment, in the test day. During the pretreatment period (days 1, 2, and 3), subjects received in the home cage a repeated and intermittent administration of a daily AMPH injection (treatment history: 0, 2, or 10 mg/kg). * $P < .05$, ** $P < .01$ for comparison vs. the SAL-history group.


PERIADOLESCENTS
ADULTS
DA
DOPAC
HVA
% of basal release
20-min blocks
Treatment History
SAL
AMPH 2
AMPH 10

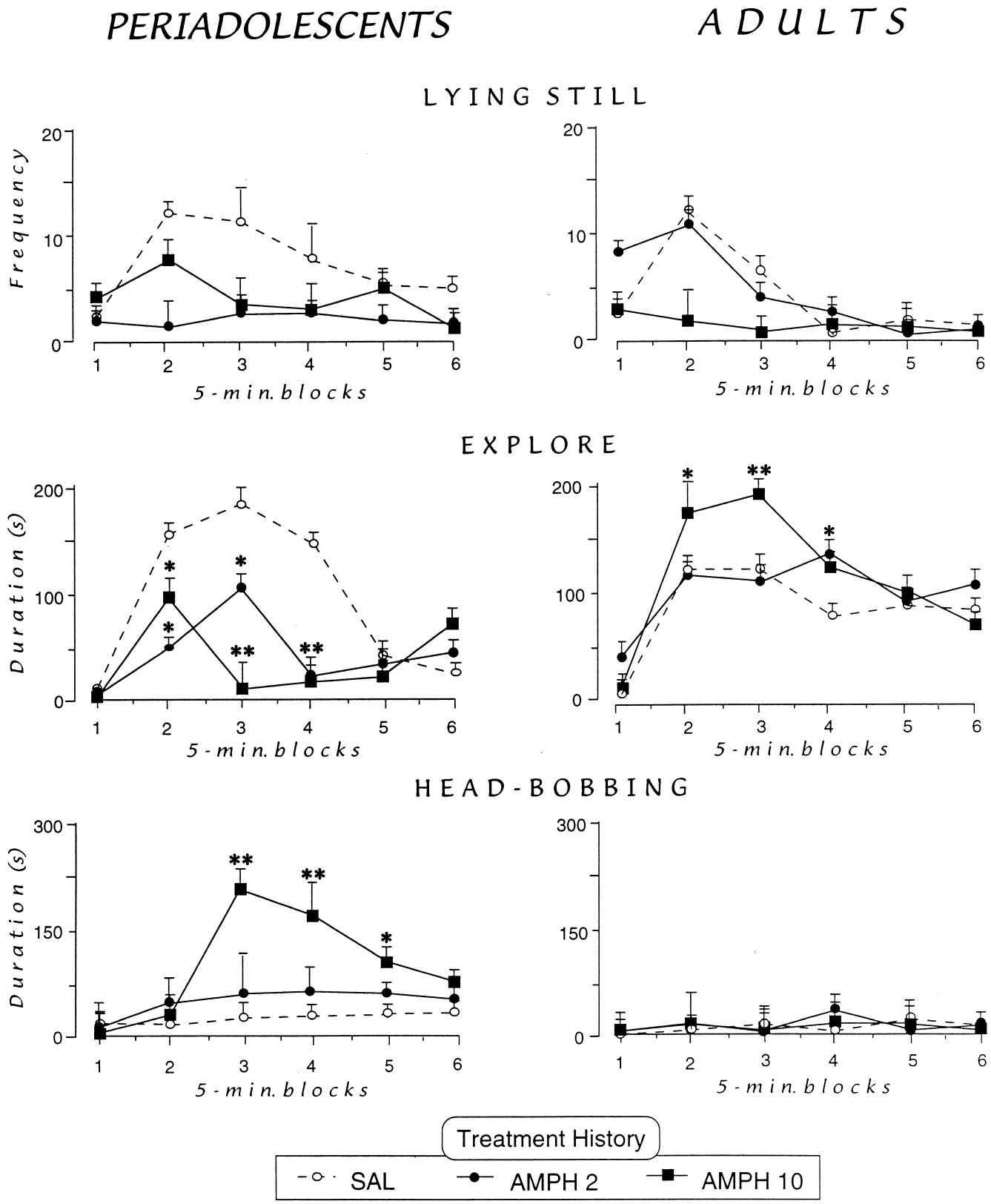


Fig. 2. Mean (±S.E.M.) frequency or duration of selected behavioral items showed by periadolescent and adult rats in response to a challenge with a standard AMPH (2 mg/kg) dose in a novel environment, in the test day. Animals were injected immediately before being placed in the apparatus. During the pretreatment period (days 1, 2, and 3), subjects received in the home cage a repeated and intermittent administration of a daily AMPH injection (treatment history: 0, 2, or 10 mg/kg). $*P<.05$, $**P<.01$ for comparison vs. the SAL-history group.

generally exhibiting during the session less episodes of *lying still* and also a lower involvement in *exploration* of the cage environment than periadolescents, Age × Time, $F(5,15) = 3.42$, 5.89, P's < .05 or less, respectively. With respect to the stereotyped *head-bobbing* behavior, levels were in general low, with a slight increment over the session in the periadolescent group, $F(5,15) = 2.88$, 6.83, P's < .05 or less, respectively (see Fig. 2).

Animals previously exposed to AMPH exhibited either sensitization or tolerance to acute drug effects as a function of the age of the subjects and of the specific behavioral item considered. As shown in Fig. 2, a previous history of repeated AMPH administration, history, $F(2,92) = 9.90$, $P < .01$, was associated both in periadolescent and in adult rats with a greater AMPH-induced suppression of *lying still* episodes when compared to SAL-history subjects, suggesting the development of a behavioral sensitization profile. Some age differences, however, emerged over the test session, Age × History × Time interaction, $F(10,30) = 2.17$, $P < .05$. As for SAL-history group, periadolescent rats showed more immobility at the end of the 30-min session than the corresponding adult group. With respect to the proactive effects of chronic AMPH administration, a marked AMPH-induced suppression of *lying still* episodes was associated with the AMPH-2 history periadolescent when compared to the corresponding adult group.

With respect to time spent in *explore/sniff* behavior, periadolescent rats administered AMPH repeatedly during the training days showed a clear-cut and dose-dependent attenuation in the response to the drug, whereas the opposite was true for the adult group, Age × History × Time interaction, $F(10,30) = 3.68$, $P < .005$. In fact, a sensitization profile was found for the latter subjects, with AMPH-10 history group showing a more marked increment of time spent in *exploration* behavior towards the middle of the session than corresponding SAL-history controls ($P > .05$ or less for the 2nd, 3rd, and 4th interval).

Stereotyped behavioral patterns, such as *head-bobbing*, were observed in response to an acute AMPH injection only in the group of animals treated repeatedly with the drug (particularly the AMPH-10 dosage) in the training days, which is suggestive of a dose-dependent sensitization profile, age, $F(1,3) = 17.94$, $P < .05$; history, $F(2,15) = 5.12$, $P < .05$. Interestingly, the finding of drug effects was limited to the periadolescent group.

3.3. Body weight

Animals were weighed immediately before each injection during the training days of the schedule, and the increment of each subject's body weight throughout the experiment was analyzed. The analysis revealed a main effect of age, $F(1,18) = 7.52$, $P < .01$, of day, $F(2,36) = 30.34$, $P < .001$, and their interaction, $F(2,36) = 16.95$, $P < .0011$. As expected, adult rats were associated with a higher body weight than periadolescents, the latter also showing the expected increment throughout the days of the experiment. A main effect of treatment history was also found, $F(2.36) = 11.66$, $P < .011$, which confirmed the expected temporary reduction in body weight in animals injected repeatedly with the high drug dose, when compared to animals from the other two groups. This profile of drug effects was, however, independent from the age of the subjects.

4. Discussion

The present results confirm and extend previous reports, which showed that sensitization to drugs of abuse can be observed in an animal model of adolescence (for review, see (Laviola et al., 1995, 1999)).

In keeping with the results of the studies reported in Section 1 (Adriani et al., 1998), a fine-grain behavioral analysis of the AMPH-induced locomotor/exploratory profile as well as stereotyped behavioral syndrome was carried out in animals of the two ages. The experimental approach, time-course analysis combined both behavioral and microdialysis investigation in the same freely moving rat. In response to acute administration of a standard AMPH dosage, important age-related differences in the sensitization profile appeared. In fact, the behavioral repertoire exhibited by periadolescents was quite different from that observed in adult rats. Specifically, the drug-induced reduction of *lying still* response appeared more prominent over the session in periadolescent than in adult rats with an AMPH-2 treatment history. Conversely, when compared to the adult group, AMPH-10 treatment-history periadolescents exhibited almost maximal levels of the compulsive *head-bobbing* stereotypy and an abatement of time spent in *exploring* the cage environment throughout the test session. This general profile can be interpreted in the context of a response competition model.

For neurochemical results obtained by microdialysis procedure in the same freely moving rat, somewhat lower levels of DA and its metabolites were seen in response to an acute AMPH challenge in periadolescent than in adult rats receiving the drug for the first time (SAL-history group). This profile is in agreement with previous studies reporting an attenuated behavioral response to an acute administration of indirect DA agonists such as cocaine and AMPH (Laviola et al., 1999; Spear and Brake, 1983). The general profile apparently does not rely on an age-specific decrease in brain-drug availability (Campbell et al., 1988), but rather appears to be related to an alteration in nervous system sensitivity (Spear, 2000; Spear and Brake, 1983). On this view, an up-regulation of postsynaptic DA receptors in rat striatal slices has been suggested to be typical of periadolescence (Bolanos et al., 1998). An overexpression of striatal DA receptors is reported to occur prior to puberty (pnd 40), receptor density decreasing to adult levels thereafter (Teicher et al., 1995). Brain areas such as the striatum

and the nucleus accumbens are thought to mature at a different pace; the neural organization reached during periadolescence being markedly different from that of adults.

The behavioral sensitization profile of adult subjects with an AMPH treatment history (namely, the AMPH-induced enhancement of *exploration* behavior as well as the abatement of *lying still* response) was apparently not correlated to any concomitant change in neurochemical parameters. The literature on this issue is mixed (see Section 1), and the latter finding was not unexpected. A number of studies have shown that behavioral sensitization to AMPH-like drugs can be obtained in the absence of an enhancement in DA release in response to the drug challenge (Kolta et al., 1985; Kuczenski et al., 1997; Wolf et al., 1992). In this framework, it should be noted that the range of dosages and the regimen of drug administration adopted in the present study (namely, once a day for 3 days) were quite mild when compared with other studies reporting the observation of biochemical and functional changes in DA systems. In these studies, the more frequently adopted regimen of drug administration included, in fact, several days of elevated drug dosages with multiple/day injections as well as extended lengths of drug withdrawal (Kalivas et al., 1993; Paulson and Robinson, 1995; Yi et al., 1990); for the role of withdrawal length in DA sensitization, see Paulson and Robinson, 1995.

More importantly, the present study demonstrated for the first time that, following a same mild regimen of chronic drug exposure, also including a brief withdrawal interval, a prominent sensitization of AMPH-stimulated DA release (see Fig. 2) was observed within the striatum area in the CNS of periadolescent rats. The latter represents a neurobiological adaptation to repeated and intermittent drug-induced stimulation of neural pathways. This profile nicely correlated with the finding of a marked behavioral sensitization (namely, the decrease of immobility and the compulsive involvement in the stereotyped *head-bobbing* behavior). These behaviors are thought to rely on drug-induced stimulation of the DA pathways in the caudate–putamen area in the CNS (Kelly et al., 1975; Staton and Solomon, 1994). On this view, an up-regulation of postsynaptic DA receptors in rat striatal slices has been suggested to be typical of periadolescence (Bolanos et al., 1998). An overexpression of striatal DA receptors is reported to occur prior to puberty (pnd 40), receptor density decreasing to adult levels thereafter (Teicher et al., 1995). These preclinical data are also consistent with clinical (human autopsy) specimens that demonstrated marked overproduction and elimination of D1 and D2 receptors in striatal areas during childhood and adolescence (Seeman et al., 1987).

With respect to mechanisms underlying sensitization, subsensitive DA autoreceptors — as a consequence of repeated drug stimulation — have been postulated to enhance DA release from drug-stimulated neuronal terminals. These changes in stimulated mesolimbic and nigrostriatal pathways could play a role in the behavioral sensitization phenomenon (Newcomb, 1985; Shalaby et al., 1981; Yi et al., 1990). In this line, a differential degree of functional maturation for DA autoreceptors in mesolimbic and striatal regions during the periadolescent period has been reported (Andersen et al., 1997; Segal and Kuczenski, 1987). This developmental phenomenon could account at least partially for the paradoxical behavioral and neurochemical findings in periadolescent rats evidenced in the present study (for a review, see Laviola et al., 1999; Spear, 2000; Spear and Brake, 1983). It is thus possible that a developmental difference in plasticity of dopaminergic function underlies developmental differences in response to repeated psychostimulant administration. Furthermore, such developmental differences could reflect anatomical differences in the ontogeny of such plasticity (Andersen et al., 1997). There are reports that the nigrostriatal feedback pathways and/or the DA transporter in periadolescents may differ from those of adult rats (Bolanos et al., 1998). Also, nigrostriatal DA neurons from immature rats are reported to be less sensitive to the inhibitory effects of cumulative amphetamine doses than neurons from adult rats (Trent et al., 1991).

On the basis of the peculiar AMPH-induced sensitization, which has been evidenced in periadolescent rats by means of both behavioral as well as microdialysis investigations, it can be tentatively concluded that subjects around this age undergo important drug-induced alterations in neurobiological target mechanisms that may make continued drug use more likely (Bolanos et al., 1998; Laviola et al., 1999). Since a similar profile is apparently lacking in adults, a certain degree of age-related increased ''vulnerability'' towards the addictive risk of these drugs might be expected during adolescence. Also, since the same neural areas within the CNS are implicated both in AMPH-stimulated motor behavior and in the modulation of drug-induced reward (Wickens, 1990; Wise, 1996; Wise and Bozarth, 1987), subjects around this age might perhaps be predicted to develop an increased sensitivity to internal states of reward following a repeated experience with psychostimulants, when compared to adult subjects (see also Yamaguchi et al., 1984).

During the period of late childhood and adolescence, neurobiological systems are still undergoing important developmental rearrangements through an integrated process of overproduction and elimination of synapses and evolution of neurotransmitter systems (for literature and discussion, see Witt, 1994). In addition, hormonal levels change dramatically during adolescence as a result of the onset of puberty. However, as outlined by Witt (Witt, 1994), the potential impact of environmental factors during adolescence, including psychoactive agents consumption, has received surprising little investigation. Yet, these factors may have a strong impact on the unique neurobiological and psycho-physiological substrate that predisposes or protects individual subjects from psychostimulant abuse and/or dependence. A better understanding of psychostimulant

effects during adolescence on the complicated interaction among genetic, neurobiological, psychosocial, and environmental factors will allow earlier and more effective prevention and treatment strategies.

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