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Acute and carryover effects in mice of MDMA (“ecstasy”) administration during periadolescence

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Abstract

In spite of the increasing evidence concerning its neurotoxicity, young human individuals are often involved in the recreational use of amphetamine-type stimulants such as 3,4-methylenedioxymethamphetamine (MDMA or “ecstasy”). A study aimed to investigate short- and long-term consequences of a repeated and intermittent MDMA administration (0, 5 or 10 mg/kg i.p., 3 days treatment history) was conducted in mice. Mice were injected at different phases in development, namely at early (28 days old), middle (38 days old) or late (52 days old) adolescence. When assessed for nociceptive response, a dose-dependent analgesia was found in middle and late adolescent mice. Carryover consequences of previous MDMA treatment were then investigated at adulthood (80 days old). In a social interaction test, levels of environment exploration and social behaviour resulted markedly increased in drug-free state as a function of drug exposure during development, whereas others behaviours were reduced. MDMA challenge (5-mg/kg dose) produced the expected hyperactivity, as well as a marked increment of hypothalamic serotonin (5-hydroxyhyptamine, 5-HT) levels. Mice treated chronically with MDMA during middle and late adolescence were associated with important reductions of the indoleamine. As a whole, these results indicate a differential long-term vulnerability to behavioural and neurotoxicant effects of MDMA as a function of the developmental stage of exposure.

Keywords: Adolescence; MDMA (3,4-methylenedioxymethamphetamine); Behaviour; 5-HT (5-hydroxytryptamine, serotonin); Long-term effect; (Mouse)

1. Introduction

3,4-Methylenedioxymethamphetamine (MDMA), popularly known as “ecstasy”, is a synthetic amphetamine derivative that has become one of the most widely used illicit substances for its psychotropic effects (Battaglia et al., 1988). Likewise, affective and psychotic disturbances are being increasingly recognised in association with MDMA abuse. Such effects are widespread at the behavioural and neurochemical level (for animal studies, see Crisp et al., 1989; McNamara et al., 1995; Lin et al., 1999; Morley and McGregor, 2000; Daws et al., 2000; Maldonado and Navarro, 2000, 2001; for human studies, see Schifano et al., 1998; Croft et al., 2001; Gerra et al., 2001; Parrot, 2001).

MDMA has been reported to be a potent and selective brain serotonin (5-hydroxyhyptamine, 5-HT) neurotoxin. MDMA administration to adult rats or squirrel monkeys results in a long-lasting decrease in 5-HT and its metabolite (5-hydroxyindolacetic acid, 5-HIAA) concentrations in specific brain regions (Ricaurte et al., 2000; Slikker et al., 1988; Ali et al., 1993). In humans, repeated MDMA usage has been correlated with the decrease of 5-HIAA in cerebrospinal fluid (Ricaurte et al., 1990; Gerra et al., 1998, 2001). Moreover, several case reports indicate the development of cognitive deficits, panic disorder and psychotic episodes often occurring as “flashbacks” after excessive MDMA use (McGuire et al., 1994; Parrott et al., 1998).

The most commonly reported positive effects of MDMA are euphoria and an increasingly feeling of closeness towards others (Peroutka, 1990). Adverse emotional effects are also commonly reported. Pathological anxiety is one such adverse effect, with several case studies reporting anxiogenic and panicogenic properties of the drug in human users (McCann and Ricaurte, 1992). In this view, studies conducted on animal models indicate both an anxiogenic and an anxyolitic profile in social interaction tests (Miczek

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and Haney, 1994; Navarro and Maldonado, 1999). However, up to date, the major part of the studies conducted on animal models have been mostly carried out on adult subjects (however, see Broening et al., 1994; Fone et al., 2002). In this framework, it must be considered that an increased risk of developing drug abuse and drug-related problems is often associated with the adolescent period, during which different patterns of temporary deviance are often observed (for a review, see Laviola et al., 1999).

In both humans and animals, adolescence is a critical period during development, which is generally associated with the development of the reproductive capacities, with individuals also acquiring mature survival skills that allow independence from parental care. Adolescence is indeed characterised by a profile of behavioural peculiarities, and increasing attention has been devoted to animal models in order to study the underlying neurobiological and neuroendocrine changes (for a review, see Laviola et al., 1999; Spear, 2000). In this view, a useful model, namely the periadolescent rodent (around 35–45 days old), has been proposed and validated by Spear and Brake (1983). Periadolescent rodents are hyperactive and particularly involved in affiliative and playful behaviours, which are important for the establishment of adult-like social relationships (Cirulli et al., 1996; Terranova et al., 1993, 1998). Subjects around this age also exhibit elevated levels of novelty/sensation-seeking behaviour together with a peculiar sensitivity to administration of psychostimulant agents (Laviola et al., 1999; Spear, 2000). As a whole, periadolescent rodents have been proposed as a useful animal model for the study of risk factors involved in the vulnerability to behavioural disorders in human adolescents (Laviola et al., 1999).

A human study conducted by Gerra et al. (1998) on adolescent MDMA users reported a modified coping response to both environmental and social stimuli, and high levels of outward-directed aggressiveness as well as elevated scores of novelty-seeking behaviour. Such behavioural profile was also associated with a dampened function of both prolactin and cortisol regulations and with a derangement of monoaminergic functions. In this view, it seemed of interest to better investigate on possible carryover effects of MDMA exposure during a critical period in development such as adolescence in a suitable animal model (see also Broening et al., 1994; Fone et al., 2002).

A first aim of this study was then to assess in mice the acute behavioural effects of a treatment with MDMA during different phases of periadolescence. A second aim was to investigate the possible long-term effects of such treatment on two different behavioural paradigms of anxiety at adulthood. These consisted of the social interaction test (File, 1980) and the novelty preference test (Laviola and Adriani, 1998).

Main effects of MDMA on brain areas have been observed at the hippocampal, cortex and striatum level (Stone et al., 1987). Yet, much less attention has been dedicated to the hypothalamic area that is very rich in serotonergic neurons (Steinbusch and Nieuwenhuys, 1981), and it also involved in core bodily functions, most of which are profoundly altered following MDMA administration. Moreover, an interaction between 5-HT function and social agonistic behaviour has been reported at the level of hypothalamus (Delville et al., 2000; see also Gregg and Siegel, 2001). Following behavioural analysis, neurochemical assessments of brain 5-HT and 5-HIAA concentrations were also conducted in the hypothalamus, a brain area.

2. Materials and methods

2.1. Subjects

Mice of the outbred CD-1 strain, without prior breeding experience, were purchased from a commercial breeder (Charles River Italy). On arrival, animals were housed in an air-conditioned room (temperature 21±1 °C, relative humidity 60±10%), with a reversed 12-h light–dark cycle (lights on at 8:00 PM). Water and food (Enriched Standard Diet purchased from Mucedola, Settimo Milanese, Italy) were available ad libitum. After a week, nulliparous females were placed with a sexually experienced male. Upon discovery of a vaginal plug (designated as gestation day 0), the females were housed individually in Plexiglas cages (33×13×14 cm).

Litters were culled to eight sibling pups with four males and four females when possible. Mouse pups were weaned on day 21 and housed in groups of four (Plexiglas cage 33×13×14 cm), according to sex.

2.2. Drug treatment

MDMA (Lipomed, Switzerland) was diluted in saline to provide appropriate doses for injections and administered i.p. in three doses: 0, 5 and 10 mg/kg, 1% of body weight. Dosages were selected on the basis of literature (Miczek and Haney, 1994; Navarro and Maldonado, 1999). Mice were randomly assigned to one of the three experimental age groups during adolescence: early (28 days old), middle (38 days old) or late (52 days old), and received a repeated and intermittent administration of MDMA for 3 days. Injections were administered in order to have a 2-day interval between each day of treatment. The drug was administered on alternate days with the purpose to reproduce the way of assumption of adolescent human users, mainly during the weekend followed by about a washout period during working days. For each age group, a vehicle (VEH), MDMA-5 and MDMA-10 group was then formed.

2.3. Apparatus and procedure

2.3.1. Nociception assessment (hot-plate test)

At the end of the chronic treatment, 1 h after being injected with the drug, periadolescent animals were assessed

for pain reactivity in a hot-plate apparatus (socrel hot-plate model-ds37: Ugo Basile, Italy) and analgesia measured as latency for fore- and hind-paw licking. Temperature was set at 55±1 °C, with cut-off being set at 100 s.

2.3.2. Social interaction test

The test was carried on male mice at adulthood (80 days old).The social interaction test involves the anxiety mice display towards an unfamiliar conspecific. Here, the long-term effects of MDMA were investigated in conditions (red dim light, unfamiliar environment) which allow the detection of both anxiolytic and anxiogenic effects (File, 1980). Mice were allocated to pairs so that each pair had received the same age–drug treatment, were of approximately equal weight and had never been caged together (n=5 pairs for each experimental group, for a total of 43 pairs).

The animals were placed in the test arena (of the same type as the home cage) for a single 15-min session. All the session was video-recorded using a professional Sony videocassette recorder VO-58OOPS apparatus. The whole session was automatically subdivided into 5-min intervals. The first and third 5 min of each session were manually scored (Observer 20, Noldus) according to an ‘‘all-occurrence’’ sampling method (Martin and Bateson, 1986) by an observer blind to the assignment of animals to the different groups. By running the tape twice, separate scores were obtained for each individual in a pair, but since the two values cannot be considered statistically independent, pair means were used for further analysis (Terranova et al., 1993; Morley and McGregor, 2000; Fone et al., 2002).

The following items of social behaviour were scored:

Attacks: the number of fighting episodes in which a mouse initiated the aggressive intercourse.
Environment exploration: sniffing the air, rearing and exploring the cage.
Social investigation: sniffing the body of the partner.
Self-grooming: self-explanatory.
Freezing: self-explanatory.

2.3.3. Novelty-seeking test

Approximately 30 days after the social interaction test, mice were assessed for novelty preference (for procedure, see Laviola and Adriani, 1998).

2.3.3.1. Apparatus. The experimental apparatus for the novelty-seeking paradigm consisted of an opaque Plexiglas rectangular box with smooth walls, subdivided into two compartments (20×14×27 cm). The connection door between the two compartments could be closed by means of a temporary partition. Mixed visual cues were associated with both compartments. One compartment had white walls and a black floor, whereas the other one had black walls and a white floor. Each compartment was provided with four infrared photobeams, placed on the wall at few centimeters from the floor. An IBM computer recorded each beam interruption eventually caused by mice. The floor and the wall of the apparatus were washed with an ethanol solution after each animal was trained or tested.

The whole experimental schedule took a total of 4 days, each subject being trained and tested between 10:00 AM and 6:00 PM. Testing of different sex and previous drug treatment groups were counterbalanced across time. Training and testing were carried out under dim illumination.

Days 1, 2 and 3: familiarisation. Animals were weighed and immediately placed for 20 min in one compartment of the apparatus, namely, the familiar compartment.

Day 4: novelty-seeking test. Animals were injected either with vehicle or with a standard 5 mg/kg MDMA challenge, and immediately after placed in the familiar compartment. After a 15-min session, the partition separating the two compartments of the apparatus was removed, and mice were thus allowed to freely explore both compartments of the apparatus (the familiar and the novel ones) for 20 min.

The following measures were obtained automatically: time spent in each compartment and locomotor activity in each compartment (number of beam interruptions/second). The whole session was automatically subdivided into 5-min intervals.

2.3.4. Assessment of serotonin (5-HT) and 5-hydroxyindolacetic acid (5-HIAA)

Immediately after the novelty preference test, all animals were transferred to an adjacent room and sacrificed by decapitation. The mice’s brains were removed, and hypothalami were collected and stored at −70 °C. 5-HT and 5-HIAA were measured by a liquid chromatographic method described previously (Bianchi et al., 1997). Briefly, the brain areas were homogenised with 0.3N HCLO4 containing dihydroxybenzylamine as the international standard. The neurotransmitter and their metabolic products were adsorbed onto alumina at pH 8.6 and then desorbed into 70 μl 0.1 N $HCLO_4$. Fifty microliters of diluted supernatant were injected onto Nucleosil C-18 reverse phase analytical column (30×3.9 cm; particle size 7 μm). The mobile phase (0.08 M citric acid, 0.04 M Na2HPO4, 0.1 mM EDTA-Na2, 0.64 mM sodium ocryl sulphate and 10% methanol, pH 3.2) was delivered at a flow rate of 1 ml/min. The eluted substances were quantified with an electrochemical detector (ESA 5100A, Bedford, MA).

2.4. Design and statistical analysis

Data were analysed using parametric analysis of variance (ANOVA) with two levels of sex, three levels of age, three levels of treatment history and two levels of challenge as between group factors (Winer 1971; Chiarotti et al., 1987). Multiple comparisons within a significant interaction were performed using the Tukey honest significant

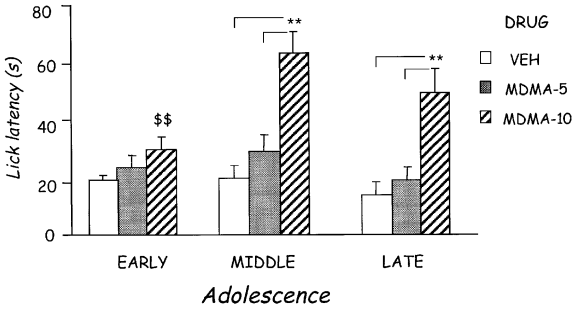

Fig. 1. Acute effects of MDMA treatment as measured in the hot-plate test (55±1 °C) during adolescence. Fore-paw lick latency (mean±S.E.M.) shown by the three age groups injected either with VEH, MDMA-5 or MDMA-10 1 h before testing. **P<0.01 between VEH vs. MDMA doses within the middle and late group.$^{\$\$}P$<0.01 between early and late groups within the MDMA-10 dose.

difference (HSD) test, or planned comparisons for each age group.

3. Results

3.1. Hot-plate test

For the latency to lick a forepaw (see Fig. 1), a main effect of acute drug treatment in the ANOVA (F(2,172)=29.39, P<0.001) revealed a dose-dependent analgesia profile in all periadolescent subjects. In the absence of significant differences in the baseline profile (VEH-injected mice) of animals belonging to the three age groups, an age-by-drug interaction was also found (F(4,172)=4.6, P<0.001). Specifically, middle and late adolescents appeared to be most sensitive to the drug treatment. Such a difference reached the significance in the comparison between middle and early group at the high MDMA dose (P<0.001).

3.2. Social interaction test

Both members of the pair received the same age–drug treatment. ANOVA was performed on pair means instead of separate scores for each individual since the two values cannot be considered statistically independent. The results for the social interaction test carried out in adult subjects are shown in Fig. 2.

ANOVA revealed a significant increase in the time spent in *Environment exploration* (left upper panel) for all subjects treated chronically with MDMA during periadolescence (main effect of treatment history, F(2,33)=4.90, P<0.05). With respect to time spent in *Social investigation* (right upper panel), previous MDMA treatment induced a bimodal behavioural profile, only a trend for treatment history (F(2,33)=2.57, P=0.09), and an age-by-treatment history significant interaction (F(4,33)=2.72, P<0.05). Specifically, subjects treated during early and late adolescence showed a dose-dependent increment (P<0.05 and 0.01 vs. VEH in the early group), whereas middle-adolescence-treated mice presented a reduction in the expression of such behaviour. Long-term consequences of an MDMA treatment history were also found in *Self-grooming* behaviour (left

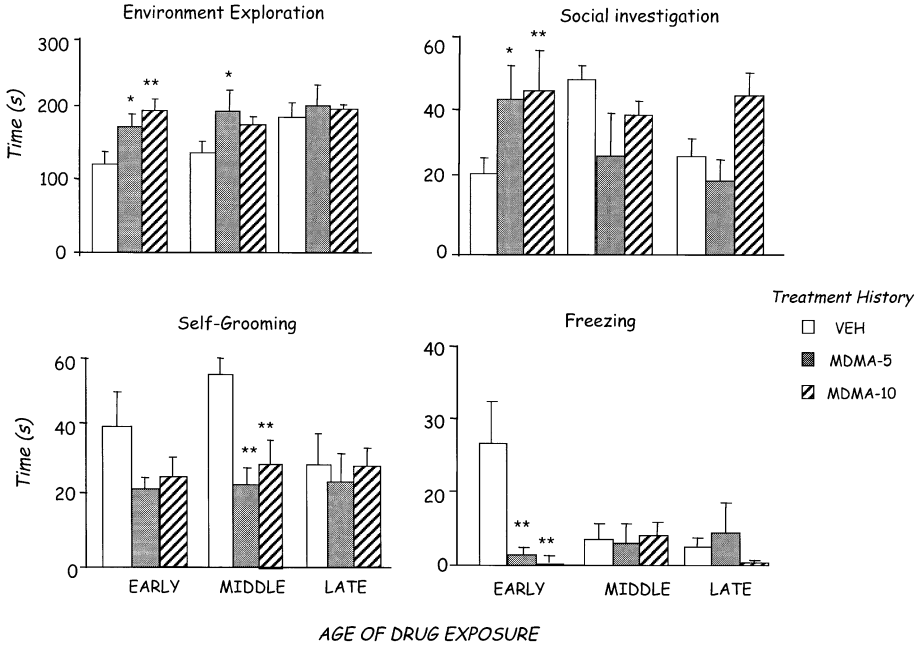

Fig. 2. Long-term effects of MDMA treatment—as measured in the social interaction test in drug-free state (single 15-min session)—shown by adult male mice of the three previous age-treated groups. *P<0.05 and **P<0.01, planned comparisons between VEH vs. MDMA doses for each age group.

bottom panel), which was reduced in all age groups (main effect of treatment history $F(2,33)=5.36$, $P<0.001$).

Aggressive items of behaviour were not affected by previous treatment with the drug (VEH=51.85 s vs. 49.6 and 60.8 s, respectively, in the MDMA-5 and MDMA-10 dose). However, time spent *Freezing* (right bottom panel) resulted markedly reduced by previous MDMA treatment during early adolescence as revealed by an age-by-treatment history significant interaction ($F(4,33)=2.82$, $P<0.05$). Planned comparisons indicated that such effects occurred specifically in the early adolescence group ($P<0.001$ vs. VEH) due to the elevated basal levels of controls.

3.3. Novelty preference paradigm

3.3.1. Familiarisation period: locomotor activity

Results are presented in Table 1. In general, a conditioned increment in levels of activity was associated with a treatment history with the higher dose of MDMA (main effect of treatment history, $F(2,184)=6.71$, $P<0.01$). ANOVA also revealed a significant age-by-sex-by-treatment history interaction ($F(4,184)=3.61$, $P<0.01$). Specifically, this drug effect was observed in males from the early adolescence group ($P<0.01$ MDMA-10 vs. VEH), but not in females. No reliable changes were found in the other two age groups.

3.3.2. Day 4 (testing day): novelty seeking (time spent in the novel compartment)

Overall, MDMA challenge increased time spent in the novel compartment in all age groups, which presented a 15% increment with respect to no-challenged subjects (main effect of challenge $F(1,19)=8.25$, $P<0.01$).

3.3.3. Day 4 (testing day): locomotor activity in the novel compartment

Overall, animals were less active in the novel compartment than in the familiar one after the partition opening (compartment, $F(1,119)=12.67$, $P<0.01$). In order to investigate possible carryover effects of the phase in development during which animals were handled and VEH-injected (no-drug history), a separate analysis was also performed. An age-by-drug challenge significant interaction ($F(2,39)=3.36$, $P<0.05$) was found. The experience of mild stress associated to the saline injection procedure on early adolescence increased the sensitivity to MDMA challenge on locomotor profile at adulthood ($P<0.05$, see Table 2). Finally, a treatment history-by-challenge interaction, which just missed significance ($F(2,125)=2.89$, $P=0.06$), was observed, indicating that a profile of sensitisation to drug effects appeared. In response to a challenge with the same drug, adult mice treated chronically during development with the high MDMA dose (10 mg/kg) exhibited consistently higher hyperactivity than other groups.

Table 1
Long-term effects of MDMA administration on levels of activity (S.E.M) during the training phase of the novelty preference paradigm

Sex	Age	VEH	MDMA-5	MDMA-10
M	early	400 (27)	430 (21)	507 (28)[a]
F		436 (19)	413 (22)	431 (17)
M	middle	416 (18)	412 (22)	388 (24)[b]
F		427 (26)	400 (27)	488 (30)
M	late	379 (32)	419 (18)	418 (21)
F		380 (28)	400 (24)	479 (39)

Locomotor activity was measured automatically as number of photobeams interruptions/second during a 30-min session/day, in adult mice of both sexes repeatedly treated during adolescence with MDMA (0, 5 or 10 mg/kg, treatment history) and belonging to early, middle or late adolescence in development. Data are collapsed over the 3 days of training. Multiple comparisons refer to the male group only.

[a] $P<0.01$ within early group.

[b] $P<0.01$ early vs. middle upon the high MDMA dose.

Table 2
Acute effects of MDMA-5 challenge in the novelty preference test

Age	VEH	MDMA-5
Early	2.7 (0.1)	3.6 (0.3)[a]
Middle	3.0 (0.2)	3.7 (0.3)
Late	3.1 (0.3)	2.4 (0.4)

Locomotor activity (S.E.M.) in the novel compartment by all age groups (namely, early middle or late adolescence): Mice were injected acutely either with vehicle or a standard MDMA-5 dose. All data are derived from mice with no previous MDMA history (vehicle-injected subjects) during adolescence.

[a] $P<0.5$ vs. VEH.

3.4. Measurement of monoamines in the hypothalamus

Results are shown in Fig. 3. As expected, acute MDMA challenge induced a marked release of serotonin as well as a concomitant reduction of 5-HIAA in all groups (main effect of challenge, $F(1,97)=32.86$, $P<0.001$ for 5-HT; $F(1,97)=41.83$, $P<0.001$ for 5-HIAA).

In order to investigate possible carryover effects of chronic drug treatment during periadolescence, a separate analysis was conducted on VEH-injected groups only. ANOVA revealed a significant main effect of treatment history ($F(2,65)=3.23$, $P<0.05$). As a whole, reduced levels

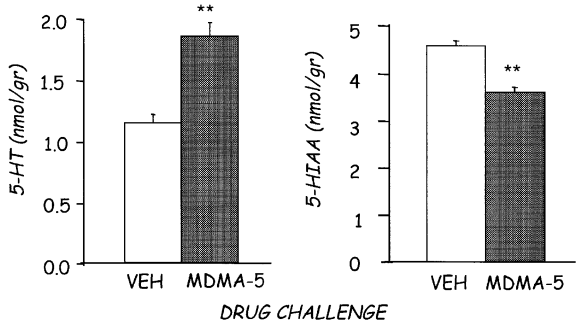

Fig. 3. Acute effects of MDMA-5 challenge on 5-HT and 5-HIAA hypothalamic levels shown by adult mice. Animals received and acute injection of either VEH or MDMA-5 and were sacrificed 1 h later. Data are expressed as nmol/g. **$P<0.01$ between VEH and MDMA-5 challenge.

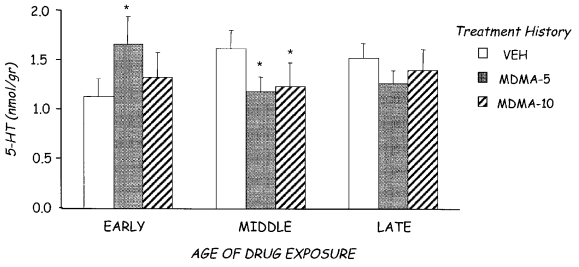

Fig. 4. Long-term effects of chronic and intermittent MDMA treatment on 5-HT hypothalamic levels shown by adult mice. Data, expressed as nmol/g, are presented as a function of age of previous exposure during development and are derived from VEH-challenged mice only. *P<0.05 planned comparisons between VEH vs. MDMA history dose for each age group.

of serotonin resulted from a previous MDMA treatment history. Interestingly, the finding of a significant age-by-treatment history interaction (F(4,65)=3.61, P<0.05) also revealed that chronic treatment with the low MDMA-5 dose during middle and late adolescence was associated with lower levels of the indoleamine at adulthood. In contrast, a chronic treatment with the high MDMA-10 dose failed to reveal any reliable differences between the age groups (see Fig. 4). On the other hand, for levels of 5-HIAA, no differences were detected as a consequence of the different treatment history (VEH=4.43 nmol/g vs. 4.43 and 4.44 nmol/g in MDMA-5 and MDMA-10 treated groups, respectively).

4. Discussion

The results of the present study can be summarised as follows:

(1) Acute treatment with MDMA induced a dose-dependent analgesia in all age groups. An elevated responsivity to drug effects appeared at middle and late adolescence.

(2) Long-lasting effects of previous MDMA treatment history were found on profile of social behaviour at adulthood. Social affiliative interactions, together with environment exploratory activity, were markedly increased. In contrast, items of aggressive behaviour were not affected by previous treatment. Early and middle adolescents appeared to be the most responsive to carryover effects of the drug.

(3) A challenge with a standard MDMA-5 dose at adulthood was associated with a profile of increased novelty seeking as well as hyperactivity in all groups.

(4) MDMA challenge induced increased levels of 5-HT concentration and a concomitant reduction of 5-HIAA in all groups. Long-term effects of drug administration during development were found on serotonin levels in the hypothalamus. Specifically, mice treated with MDMA during early and middle adolescence exhibited a marked reduction in the 5-HT concentration in the hypothalamus at adulthood.

MDMA produced a number of clear effects in the battery of tests, although very different outcomes were associated with different tests. In particular, MDMA had an analgesic effect in the hot-plate test, a prosocial effect in the social interaction test, and stimulant and anxiolytic effects on reactivity to a novel environment. Effects of a chronic treatment with MDMA during adolescence were persistent throughout adulthood, and a differential response to MDMA as a function of the age of first administration with such a drug was also observed. This indicates that long-lasting effects were associated mainly with a very precocious MDMA treatment (see also Broening et al., 1994; Fone et al., 2002). In this view, our results support adolescence as a crucial ontogenetic period during which maturational rearrangements can exert a strong influence on the differential vulnerability to psychostimulants (Laviola et al., 1999; Spear, 2000).

During periadolescence, acute administration of MDMA induced a dose-dependent elevation in pain threshold in all age groups. Our results are in agreement with literature data indicating a dose-dependent analgesic effect of MDMA on the hot-plate test (Nencini et al., 1988; Crisp et al., 1989), and a role for 5-HT regulations in this profile. In line with these data, we found a differential sensitivity to nociception in the three age groups. Specifically, administration of MDMA at later age was associated with a much higher reduction of sensitivity to pain stimulation. Although the profile is mixed, differences in sensitivity to acute MDMA found in the three age groups could be accounted by a differential degree of maturation of the serotonergic system as a function of the stage of development. Indeed, data concerning the ontogenesis of the serotonergic system indicate that in the rat, such a system undergoes developmental discontinuities during time periods that may, in some cases, include adolescence. In this view, estimates of 5-HT turnover in the striatum area have been reported to be approximately fourfold lower in middle adolescent (P30–P40) rats relative to younger and adult (P60–80) animals (Teicher and Andersen, 1999).

With respect to long-term effects of MDMA treatment, developmental exposure to such a drug during early and middle adolescence resulted in marked increments at adulthood in levels of spontaneous locomotor activity and of environment exploration. Interestingly, this profile was specifically associated with a high MDMA treatment history during early adolescence. Also, a sex-related vulnerability appeared with males and not females being affected, although the gender issue in sensitivity to MDMA effects is, however, mixed (Slikker et al., 1988 for rats; for a review on human, see Parrot, 2001). Early-treated subjects showed at adulthood also increased social investigation in a dose-dependent manner (see Fig. 2, upper right panel) (for contrasting results in rats, see, however, Fone et al., 2002). These results about a prosocial and anxiolitic effect of MDMA are in agreement with a previous study by Morley and McGregor (2000) reporting that when given acutely, MDMA actually increases social interactions (but see also Navarro and Maldonado, 1999; for human studies

on acute MDMA and social behaviour, see Peroutka, 1990). Interestingly, the same authors have shown in a very recent study (Morley et al., 2001) that social and exploratory behaviour are on the other hand markedly reduced when assessed after 3 months following a repeated MDMA administration at adulthood. With respect to our results, it is interesting to note that long-term effects of MDMA on patterns of social behaviour are very much different depending on the developmental phase during which drug administration has occurred (adolescence vs. adulthood).

Finally, data from the neurochemical assessment indicated that, as expected, acute challenge with MDMA increased the release of serotonin in the hypothalamus. Interestingly, a previous treatment with the same drug during adolescence had long-lasting effects—namely, a reduction in 5-HT levels—which was more marked in mice treated precociously (early adolescence) than later in development. A study by Stone et al. (1987) indicated that administration of multiple doses of MDMA in the rat over a short period resulted in significant decreases in serotonergic parameters for up to 110 days after treatment in hypothalamus, striatum and hippocampus. The reported neurotoxicity of MDMA on serotonin pathways exhibits some species specificity, as comparable decreases in cerebrocortical 5-HT, 5-HIAA and 5-HT uptake sites have been observed in rat and guinea pig, whereas no significant changes in any of these serotonergic parameters have been detected in mouse brain (Battaglia et al., 1988). However, transient alterations in biogenic amines and metabolites after MDMA administration have been reported also in mice (see Steele et al., 1989). Apparent inconsistencies in our study with the reported MDMA influence on 5-HT system could be due to a reduced vulnerability in mice with respect to rats for this parameter. Mixed results are, however, available, such as the absence of serotonergic neurotoxicity in adult rats in several brain areas following developmental MDMA administration (Fone et al., 2002).

The neurochemical investigation of our study addressed to the hypothalamus (see Introduction). Indeed, a wider regional assessment could have been certainly useful. Furthermore, MDMA is also reported to produce in mice long-term effects on dopaminergic system (see Colado et al., 2001). In this view, further investigations of this laboratory aim to replicate the results obtained in the present study with the addition of a multiple brain regions analysis of MDMA-induced changes, and of other neurotransmitters changes in the hypothalamic area in addition to serotonin.

As a whole, our study suggests a long-lasting differential vulnerability to the effects of ecstasy as a function of the sex of the subject and developmental stage of first approach with such a drug. A very recent work by Adriani et al. (in press) indicated a peculiar vulnerability to nicotine oral consumption in mice during early adolescence. In this framework, the earlier a crucial period of vulnerability to drugs of abuse is detected during development, the more efficiently an anticipatory and therapeutic strategy can be adopted.

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