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# Psychobiological risk factors for vulnerability to psychostimulants in human adolescents and animal models

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**Abstract**

Adolescence is associated with an increased risk of developing drug abuse/dependence. During this ontogenetic phase, brain and hormonal systems are still undergoing crucial maturational rearrangements, which take place together with significant modifications in psychosocial development. However, the neurohormonal and behavioral facets of adolescence have been poorly investigated in relation to the vulnerability to psychostimulants such as MDMA ("Ecstasy") and amphetamine (AMPH). Novelty-seeking, a temperamental/behavioral trait that is typical of this age period, might substantially contribute to both psychological and psychobiological vulnerability. In humans, an elevated score of novelty-sensation seeking and a derangement of monoaminergic function were both associated with late adolescence MDMA users compared to controls. In animal models of periadolescence, the search for novel stimuli and sensations actually shares a common neurobiological substrate (the reward-related brain mesolimbic pathways) with psychostimulants. The present review summarises recent work in mice, which indicates that periadolescent subjects are characterized by an unbalanced and "extremes-oriented" behavior and by elevated novelty-seeking compared to adults. Repeated and intermittent administration of cocaine or AMPH was associated with the development of a prominent locomotor sensitization in periadolescents, which failed to exhibit the marked sensitization of the stereotyped behavioral syndrome—possibly associated with poor welfare—that was typical of adults. A unique profile of integrated behavioral and physiological hyporesponsivity to both forced novelty and acute AMPH administration during periadolescence was also found. As a whole, these results, together with previous work on this topic, suggest that periadolescents may be more "protected" from AMPH-related aversive properties, and perhaps more vulnerable to the experience of internal states of reward, than older animals. Thus, the present animal model of adolescence seems to represent a reliable and useful method for the investigation of vulnerability to a variety of habit-forming agents or emotional experiences whose positive reinforcing properties may rely on common neurobiological substrates. A deeper understanding of psychostimulant effects during adolescence on the complex interaction between genetic, neurobiologic, psychosocial, and environmental factors will lead to earlier and more effective prevention and treatment. 

*Keywords:* Developmental plasticity; Periadolescence; Animal models; Individual vulnerability; Ontogeny of behavior; Rodents; Novelty seeking; Incentive motivation; Stereotypies; Behavioural sensitization; *d*-Amphetamine; Cocaine; Ecstasy

## 1. Introduction

Even if most people occasionally come into contact with psychoactive substances, it is clear that not all start a regular use of these compounds. Indeed, the identification of risk factors that might enhance or reduce the risk of developing drug dependence and related problems is very important in order to reduce the prevalence of these problems in the population. Risk factors are those characteristics of the person or the environment that are associated with an increased probability of maladaptive developmental outcomes (for literature and discussion, see Ref. [1]). In this context, drug use seems to be one of the several coping responses that can be used when the individual is exposed to an increasing number of vulnerability conditions [2,3]. For these reasons, considerable research effort has been devoted to the study of risk factors that make some individuals more vulnerable than others. These individual differences may be traced back to either genetic or environmental factors, and most reasonably to an interaction between the two (see, e.g. Refs. [4,5]).

Recent research has suggested that, within the same subject, vulnerability also varies with age. Specifically, an increased risk of developing drug abuse and drug-related problems is associated with the adolescent period, during which different patterns of temporary deviance are quite often observed [1]. Indeed, epidemiological data suggest that the use of various kinds of psychoactive agents is

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widespread during this period, ranging in a strict sense from 11–12 to 17–18 years of age [6]. Adolescents are likely to start with tobacco and/or alcohol, followed by marijuana, and eventually psychostimulants and/or opiates (see Ref. [7]). Adolescent involvement with drugs seems to be multiply determined: exposure to a higher number of risk factors is not only a reliable correlate of drug use, but it also increases drug use over time, implying a true etiological role [1,3,8].

Adolescence is a unique ontogenetic period during which plasticity of the brain continues through neuroanatomical, neurochemical, and neurophysiological processes. Corresponding to shifts in brain maturation are significant transitions in cognitive, psychological and social development, as well as age-specific alterations in behavior and in psychopharmacological responsivity [3,9–11]. Thus, environmental influences during adolescence are bound to interact with unique neurobiological and psychosocial strengths and weaknesses to predispose or protect an individual from drug abuse and/or dependence. 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, and very little is known about the unique effects and consequences which the exposure to potent psychoactive agents may have during this developmental period. However, recent epidemiological research has shown that individual differences in the age of first contact with psychoactive compounds may influence subsequent patterns of drug use as well as the development of drug-related problems. Indeed, certain patterns of consumption, as well as the likelihood to shift from use to abuse and the chances of developing dependence, all seem to be positively correlated with an early approach to drugs (for literature see Refs. [8,12,13]). An early onset of drug use might have such effect by disrupting developmental processes that lead toward successful adaptation during adolescence. Acute or chronic drug exposure could interfere with normal growth, maturation, as well as the development of cognitive and psychosocial competence.

The studies illustrated in this paper will focus on age-specific neurobehavioral function in adolescents, placing particular emphasis on developmental and experiential factors which contribute to drug use and abuse (often using synthetic psychostimulants as an example). The intention is to provide evidence of the importance of the assessment of adolescents per se for this kind of research. Indeed, the continued integration of research on normative and deviant development during adolescence has proven to be useful to the field, as research on normative and clinical populations can inform each other. Furthermore, the integration of longitudinal and laboratory experimental research, including the integration of research with animal and human populations, should provide a more complete and rich understanding of adolescent development and vulnerability.

A particular temperamental trait, which has been put forward in both epidemiological and experimental studies, is novelty or sensation seeking. According to Zuckerman [14], such a trait is characterized by “the continuing necessity to experiment various, novel and complex sensations”, which are hypothesized to be rewarding. Involvement in risky and stressful activities that are avoided by others, as well as in illicit drug use, has been shown to be more prevalent in individuals showing elevated scores on this trait [15]. Indeed, in a review by Arnett [16], human adolescents are reported to be statistically over-represented, when compared to adults, in the group showing a prominent motivation towards seeking novel sensations. Thus, in human adolescents, the continuing search for sensations and the need for novel stimuli may account, at least partially, for the elevated level of “curiosity”, and for the first approach, towards the experience of psychoactive agents.

Furthermore, sensation seeking has been associated with all three groups of traits (impulsivity, aggression, as well as approach and reward seeking) which the biochemical model developed by Zuckerman (for literature, see Ref. [17]) describes as related to three specific monoamine neurotransmitters, dopamine (DA), norepinephrine (NE), and serotonin (5-HT). These neurotransmitters have long represented primari foci for the investigation of the biological bases of fundamental behavioral mechanisms such as approach, inhibition, and arousal. Other psychobiological models of personality, proposed by authors such as Depue, Cloninger or Gray (for literature and discussion, see Ref. [17]) have also emphasized the relationships between basic behavioral mechanisms in animals and humans, personality traits in humans, and the monoamine neurotransmitters. Furthermore, recent advances in molecular genetic research may hold the promise of more direct and definitive findings linking neurotransmitters, hormones and their regulators to personality traits in humans and behavioral traits in other species. The notion that novelty-seeking really has a basis in genetic abnormalities of the DA receptor has recently been confirmed by genetic studies [18]. It was shown that subjects carrying a particular allele of the D4 receptor had higher scores in novelty-seeking than those with the normal genotype.

The studies reported by Netter and associates [19] represent a major contribution to this literature. Relevant to the role of physiological parameters (such as levels of the stress-related hormone cortisol) in sensation seeking, the high sensation (experience) seekers demonstrated a blunted response to a 5-HT agonist (ipsapirone), indicating a weaker serotonergic reaction to the drug. Indeed, the novelty-seeking subjects have been found to present a 5-HT deficit and a derangement in monoaminergic systems which is probably independent from chronic substance abuse [17]. As such, the relationship between novelty-seeking levels and hormonal parameters in response to 5-HT system stimulation was specifically investigated—in the clinical laboratory of one of our group [2]—as a function of the past history with psychostimulants (see below).

It is notable that, in spite of the scientific evidence

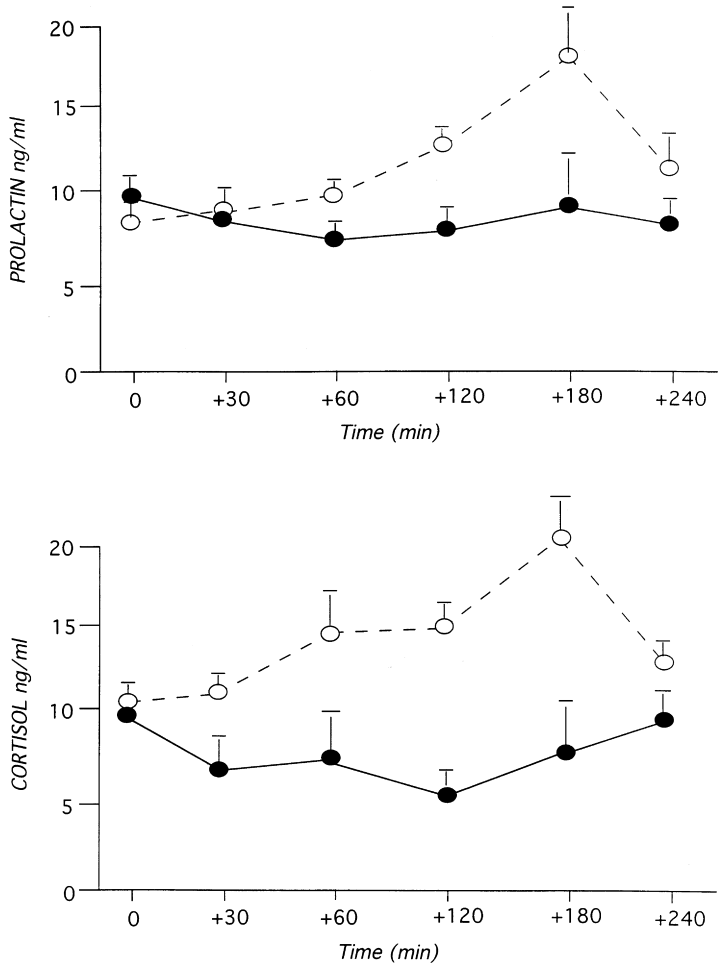


Fig. 1. Time course of prolactin (upper panel) and cortisol (lower panel) responses to *d*-fenfluramine stimulation (mean ± SE) in ± 3,4-methylene-dioxymethamphetamine (MDMA) late adolescence users (closed circle) and in normal volunteers (open circle). ($N = 15$). (Reprinted with permission from Ref. [2].)

concerning its neurotoxicity [20,21], young human individuals are increasingly involved in the recreational use of amphetamine-type stimulants such as MDMA (or "Ecstasy"), a synthetic psychostimulant which exerts its effects by massive releasing of both serotonin and dopamine from nerve terminals. MDMA (3,4-methylene-dioxy-methamphetamine) effects on the central nervous system have been widely investigated in experimental animals [22,23]. Dopamine synthesis inhibitors have also been found to induce significant changes in ecstasy effects [24], supporting the hypothesis of an involvement of the dopaminergic system in MDMA action [25] which may underlie its rewarding properties [26]. These drugs are used in dance parties, "rave" parties and after-hour dancing with techno-music, in the attempt to decrease fatigue perception and increase endurance [21,27,28]. The expected MDMA effects, reported by ecstasy users, include mood improvement, closeness, sensual and perceptual enhancement, gained insight and general activation [29,30], suggesting that this amphetamine-type stimulant exerts also in humans a variety of influences on the monoaminergic pathways normally involved in emotional behavioral and physiological changes [20,31,32].

The relationships between temperamental features, such as impulsivity and aggressiveness, and the use of this specific psychostimulant agent are still poorly understood in the few experimental protocols in humans [26], as are the biological and psychological consequences of chronic use of MDMA. For this reason, in a very recent clinical investigation [2], a number of late adolescence MDMA users, who did not show other drug dependencies or alcohol abuse and who had not used other drugs for prolonged periods, underwent a series of neuroendocrine and psychometric tests. Their responses were compared to healthy NON-USER controls in a test of mental stress as well as a drug challenge with *d*-fenfluramine (a specific serotonergic stimulus). Many clinical studies had previously shown that hormone responses to neurotransmitter challenges are suitable tools for investigating the sensitivity of 5-HT and DA receptors. The results obtained indicate that MDMA users, who were evaluated at least three weeks after MDMA discontinuation, were characterized by significantly reduced prolactin and

Table 1
Psychometric assessment.

Values are expressed as means ± SEM. *$p < 0.05$ vs control.

Personality was investigated by Minnesota Multiphasic Personality Inventory (MMPI) and by the Personality Diagnostic Questionnaire-Revised (PDQ-R). Characters and quantification of aggressiveness (defined as direct, indirect or verbal, as irritability, negativism, resentment, suspiciousness and total score) were analyzed by the BDHI, Buss-Durkee Hostility Inventory; Depression was monitored by the Hamilton Rating Scale for Depression (HRS-D). All the subjects ($N = 15$) have been submitted to the Tridimensional Personality Questionnaire (TPQ) to investigate the temperamental aspects, and particularly "harm avoidant", "novelty seeking", "reward dependent". (Reprinted with permission from Ref. [2].)

| | MDMA subjects | Control subjects |
|---|---|---|
| MMPI-depression | 64.3 ± 3.7* | 48.5 ± 3.2 |
| PDQ-R total Score | 33 ± 2.4 | 17 ± 1.1 |
| BDHI total score | 62.8 ± 5.7 | 55.6 ± 4.9 |
| BDHI direct | 57.1 ± 4.3* | 42.5 ± 3.9 |
| BDHI guilt | 58.4 ± 6.2* | 41.8 ± 4.0 |
| HAMD | 14.9 ± 3.4* | 5.1 ± 2.2 |
| TPQ novelty seeking | 27.9 ± 5.3* | 16.7 ± 3.1 |
| TPQ harm avoidant | 18.3 ± 5.7 | 12.0 ± 4.1 |
| TPQ reward dependent | 11.5 ± 3.9 | 13.4 ± 3.0 |

cortisol responses to drug challenge, as well as by a combination of depressive pattern, dysphoria, high levels of outward-directed aggressiveness and elevated scores of novelty-seeking (sensation-seeking/risk-taking) behavior (see Fig. 1 and Table 1). It was therefore concluded that a psychostimulant such as MDMA, which exerts its effects by massive release of both serotonin and dopamine from nerve terminals, could arguably have been chosen by these individuals as an unconscious self-medication for an underlying dysfunction in monoaminergic (mostly 5-HT) pathways (see also Ref. [17]).

The studies reviewed here clearly illustrate the fruitfulness of a comparative approach for understanding brain–behavior relationships. Indeed, we can study behavior in both humans and animals and attempt to look for common biological correlates. Novelty-seeking and positive reactions to novel situations have been an essential part of the definition of sensation seeking from the start, and have provided a basic model in animals as well as in young humans. The model proposed by Zuckerman (for literature, see Ref. [17]) was initially developed through psychometric and behavioral studies in humans. However, current advances are largely based on studies of other species such as rats, cats and monkeys, and correlational and experimental data from both animal and human studies tend to support the model. A comparative approach toward the study of sensation seeking has long been advocated—with an emphasis on exploratory behavior in novel situations, approach to novel stimuli, sociability and sexual and consummatory behavior, as well as on drug self-administration—as a useful means to investigate the genetic and biological basis of the trait [14].

Some behavioral and physiological characteristics have been described both in rats and mice which resemble some of the features found in human high-sensation seekers. Environmental exploration represents a fundamental aspect of the behavioral repertoire, and has both ontogenetic and phylogenetic significance. Unlike other vertebrates, mammals are capable of coordinating the disparate information from different senses to create in the brain a model of the real world [33]. This picture of reality is established in childhood and requires regular revision in the light of changes that occur in the animals experience and surroundings. Thus, mammals are essentially information seekers, and are biologically designed to pay more attention to novel information than to the familiar. They actually seem to be both attracted to and activated by novel stimuli as well as by variations in the setting or intensity of familiar ones [34,35]. Experimental evidence indicates that the experience of novelty is associated with activation of the mesolimbic dopaminergic system in the CNS; for example, entering a novel environment in rats is associated with an elevation of dopamine levels within the nucleus accumbens [36]. Furthermore, lesions of this area, induced by 6-OHDA, block the expression of novelty-seeking behavior [37,38]. Indeed, these same brain areas are involved in reward-related phenomena induced by salient natural stimuli as well as by drugs of abuse such as psychostimulants [39–42]. Consequently, the satisfaction of a novelty-stimulated "curiosity" seems to have most of the characteristics of natural rewarding events [43].

Over the last few years, we have investigated age-related differences, among several other facets of the behavioral repertoire, in exploratory behavior of both rats and mice, in an attempt to identify the differential behavioral and physiological characteristics of age-related discontinuities in behavioral reactivity to novelty. Our findings raise the question of the nature of the behavioral and biological relationships and suggest some general theoretical considerations.

## 2. Novelty-seeking and periadolescence

As suggested above, the identification and thorough characterization of critical ontogenetic periods associated with increased basal levels of novelty-seeking and, perhaps, by increased biological vulnerability to the effects of drugs abuse, could have great psychobiological and clinical–therapeutical importance. Human sensation-seeking scores have been reported to decline with age, after having reached a peak in late adolescence [14]. However, the experimental evidence on the interaction between psychostimulants and novelty-seeking in animal models is mixed, and mainly derives from the study of adult subjects [44,45]. In fact, although an age-related decline of novelty-seeking scores has also been found in rats [4], no systematic studies of the topic are available on periadolescence, defined as the ontogenetic period that encompasses the 7–10 days preceding the onset of puberty (at about 40 days of age in rats and

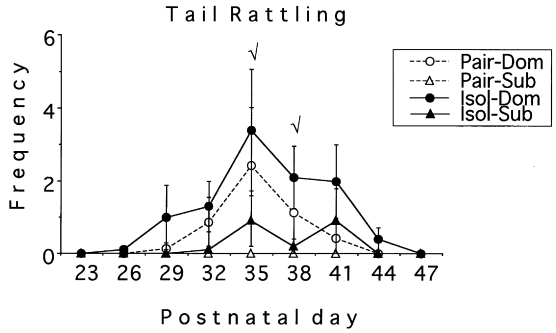


Fig. 2. Mean frequency ( ± SEM) of the ambivalent behavior *Tail Rattling* recorded (single 30-min session) during development in dominant (DOM) and submitted (SUB) mice, kept in either paired (PAIR) or individual (ISOL) housing. Social encounters were conducted between experimental dyads of initially unfamiliar subjects of the same housing condition, every third day, from pnd 23 to 47. $N = 7$–10 subjects in each final experimental group. Post-hocs: DOM vs SUB on PNDs 35, 38 ($p < 0.05$). (Reprinted with permission from Ref. [52].)

mice), and the first few days thereafter [11]. The investigation of an animal model of adolescence was thus felt necessary.

Periadolescent rats and mice are reported to be hyperactive according to several behavioral measures [9,11,46]. However, as evidenced by Spear and associates, "hole-poke" behavior, a measure of exploratory motivation—which is largely independent from the animal's gross locomotor activity—is expressed more frequently and for a longer time by 35-day-old rats compared to younger and older animals. From a social perspective, animals around this age are characterized by a pervasive expression of the affiliative and playful components of the behavioral repertoire [47–53]. In particular, Panksepp [49] (see also Refs. [48,53]) reported that the ontogeny of rat social play is characterized by an inverted-U-shaped function, with a peak between 32 and 40 days of age. According to Williams and Scott [54], a crucial period for social development may occur around this age, when adult-like fighting develops in association with the pubertal surge in androgens [55–58]. Terranova and colleagues [52], as shown in Fig. 2, observed that the period around postnatal day (pnd) 35 also corresponds to an important phase in the determination of a clear-cut differentiation in the social roles of mouse pairs (i.e. formation of adult-like dominance/submission relationships), with a high expression of the "ambivalent" *Tail Rattling* behavior that is typically elicited in contexts of uncertainty and contains both threatening and fleeing components [59–61].

These behaviors, which correlate only loosely with simple activity measures, are functionally similar, occur only in the presence of conspecifics and, most interestingly for our purposes, they follow a similar ontogenetic trend. It has been hypothesised by several authors that such a specific behavioral profile may be adaptive for the particular "ecological niche" of periadolescent animals, facilitating the expression of exploration and sociability (for literature and discussion see Refs. [11,47,50,62]).

### *2.1. Novelty-seeking (free-choice paradigm) and acute AMPH effects*

Consistent with the above considerations, a first study in mice investigated the interplay between a particular natural willingness to search for novel stimuli and the effects of repeated and intermittent *d*-amphetamine (AMPH) administration. Indeed, according to Bardo and colleagues [63], who recently reviewed the evidence for a psychobiological association in animal models between the tendency to sensation/novelty-seeking and the willingness to use psychostimulant drugs, all these experiences share a common neurobiological substrate, namely the activation of brain meso-limbic dopaminergic pathways [36] (for human studies, see Refs. [18,64]). The latter pathways have also been consistently related to reward-related phenomena [39,41] (for a review see Refs. [40,42]).

Following repeated association between psychostimulant drugs and a distinct environment, contextual cues acquire the ability to elicit a conditioned approach response in rodents (for literature, see Refs. [65,66]). To shed more light on the nature of the above processes and underlying neurobiological mechanisms, an experimental procedure was designed [67], which allowed both familiarization to one specific compartment of the apparatus and repeated associations between AMPH and this environment. During this Training period, adult (pnd $> 70$) and periadolescent (pnd 33–43) mice were randomly assigned to three different treatment history groups, which were injected with different doses of AMPH (0, 2 or 10 mg/kg i.p. once/day) for three days in a familiar environment. To investigate the potential carry-over effects exerted by repeated AMPH-induced stimulation on later responses to natural rewarding events (such as novelty), the animals were tested following a 48 h-wash-out period from the last drug injection in a free-choice novelty preference paradigm by placing them on Testing day in the familiar and pretreatment-paired environment. They were also challenged with either saline (SAL) or a standard AMPH dose (2 mg/kg), to assess the acute effects of the same drug on novelty-seeking performance. In agreement with previous reports [44,45,68], on Testing day, when mice were allowed to freely move from the familiar to a completely unknown environment (the novel side of the apparatus) (see Fig. 3, upper panel), all subjects showed—irrespective of previous treatment history—both an increased arousal (data not shown) and a marked preference for the novel environment. However, consistent with our hypothesis, periadolescent mice spent a significantly higher percentage of time in the novel compartment, when compared to adult subjects, suggesting a higher novelty-seeking trait to be characteristic of this age. This approach response has been considered as indirect behavioral evidence of an internal state of reward, because it is

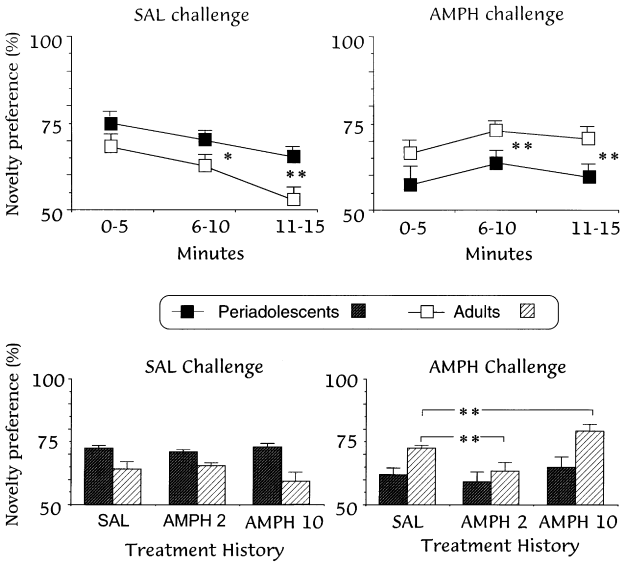


Fig. 3. Novelty-seeking as measured by the mean ( ± SEM) percentage of time spent in the novel compartment by subjects of both ages on testing day. During the Training period (days 1, 2 and 3), mice received a daily AMPH injection (treatment history: 0, 2, or 10 mg/kg) immediately before being placed for 20 min in the familiar compartment. On Testing day, animals were challenged with either SAL (left panel) or a standard AMPH dose (2 mg/kg, right panel) and placed in the drug-paired compartment. After 20 min, a partition was removed and mice were allowed free access to a novel compartment of the apparatus for a 15-min session. Upper panels: the behavioral profile over the session is presented as a function of the age of the subjects. Lower panels: data are presented as a function of the age and treatment history of the subjects. $^{**}p < 0.01$ ($n = 10$). (Reprinted with permission from Ref. [46].)

unconditionally elicited by a number of rewarding natural stimuli [69]. Interestingly, when spending time in the novel environment, periadolescents seemed to express somewhat higher levels than adults, suggesting a more marked profile of novelty-induced arousal to be typical of this age (for literature and discussion, see Ref. [46]).

These results were not unexpected, on the basis of human data reporting that novelty-seeking is typically high during adolescence, and undergoes a subsequent decrease as subjects grow older [14]. However, the profile obtained provides some confirmation of the validity of the present animal model, and suggests its utility as an experimental paradigm. From an eco-ethological perspective, it is during the periadolescent period that rodents are seen to leave the nest and begin to explore at some distance from the nest site [62]. Thus, elevated levels of novelty-seeking and novelty-induced arousal seem to be highly adaptive for animals around this age. In the context of the present study, the experience of novelty can also be thought to have a somewhat higher rewarding value for periadolescents than for adults, leading to the hypothesis that the CNS regulatory systems underlying novelty-seeking behavior are set at a different basal level in the two age groups (see below).

The available literature on the effects of acute AMPH administration in novelty preference paradigms is mixed, in that, whereas such a challenge is reported to have no effect in adult rats [44], a dose-dependent reduction of novelty preference has been found following the administration of metamphetamine in adult mice [45]. No direct comparisons with these reports are possible, due to important methodological differences. However, the data reported here clearly indicate that the AMPH challenge had quite opposite effects depending on the age of the subjects (see Fig. 3, upper panel), strongly increasing novelty seeking in adults while decreasing it in periadolescents. It has been previously shown that response to AMPH is strongly dependent on the baseline level, because it enhances the response when the baseline is low, but reduces it when the baseline is high [70]. As we have seen in the present experiment, animals of the two ages actually showed different baseline novelty-seeking profiles, consistently lower in adults compared to periadolescents. Thus, a higher threshold for the incentive stimulation induced by novelty might be hypothesized for the adults, so that acute AMPH stimulation would be needed in this group in order to sensitize CNS systems underlying novelty-seeking behavior and therefore allow them to reach the elevated levels of novelty preference shown by drug-free periadolescents. On the contrary, when the latter subjects were injected with AMPH, the over-stimulation of drug-targeted CNS systems might have produced a marked reduction in the active search for novelty, which apparently lost its incentive properties. As a whole, the different baseline levels expressed by animals of the two ages, as well as the age-related differences in the response to the AMPH challenge, could be interpreted as evidence of a shift to the left in the inverted-U shaped profile of drug response during periadolescence.

### *2.2. AMPH-conditioned incentive properties*

In this experiment, the unconditioned novelty-related motivation towards an unknown environment was directly compared with the drug-conditioned incentive motivation for a familiar and pretreatment-paired one (conditioned place preference, CPP procedure) [67]. With respect to the carry-over effects of each animal's history of AMPH treatment, and in the absence of differences within the group acutely injected with SAL, significant carry-over effects of the past experience with the same drug on the time spent in the novel environment were found upon AMPH stimulation (see Fig. 3, lower panel). In fact, within the adult group, mice with an AMPH 2 treatment history showed significantly lower levels of novelty preference than SAL treatment history controls. Consistent with a previous report [67], these results suggest that AMPH-conditioned incentive properties, experienced in and associated with the familiar compartment during the Training period, were able to devalue the strength of the unconditioned motivation towards novelty on the Testing day. An opposite profile was found for adult mice with a treatment history of AMPH 10, which spent significantly more time in the novel environment

compared to mice from the other two groups. In this case, it can be hypothesized that an excessive arousal (for a possible role of drug-induced stereotypies see also Fig. 6, bottom panel) induced by an elevated AMPH (10 mg/kg) dosage on Training day 1 produced a conditioned place aversion for the familiar drug-paired compartment, so that when offered a free choice, the mice took refuge in the other one [67] (see also Refs. [66,71]).

Conversely, no carry-over effects of treatment history were found in the periadolescent animals. To explain why this group did not develop an adult-like conditioned place aversion for the familiar compartment repeatedly paired to AMPH 10, it should be noted that an age-related difference appeared in the expression of stereotyped patterns of behavior on Training day 1. In fact, following acute AMPH 10 administration, only adult subjects exhibited elevated levels of stereotyped behavioral activity. This raises the possibility that periadolescent animals did not experience the negative correlates (see below) of AMPH effects (see, e.g. Refs. [71,72]). Thus, as a function of the drug dosage and of the age of the subjects, differential positive or negative incentive properties appear to be evoked by the AMPH-conditioned environment.

In response to the AMPH administration, rodents generally show either a profile of locomotor hyperactivity or a stereotyped behavioral syndrome, as a function of drug dosage (see, e.g. Refs. [66,73,74]). Locomotor hyperactivity is produced by certain dosages of AMPH, as well as by other drugs of abuse [73]. AMPH-induced release of dopamine within the nucleus accumbens is considered to be involved in such behavioral change [74]. As the same neural substrate seems to modulate both unobservable subjective reward and measurable locomotion, the AMPH-induced behavioral hyperactivity has been considered as an indirect index of reward [75], resembling the "euphoria" induced by this drug in humans. Behavioral sterotypies have instead been proposed to serve as a coping mechanism for drug-induced excessive arousal [76,77]. Because of such a "poor welfare" experience, stereotypies might also underlie potential AMPH-related aversive properties [66,71,72,78]. Results derived from the detailed behavioral analysis carried out in the present study seem to be consistent with these hypotheses. In fact, within the AMPH challenge group, a clear-cut and dose-dependent sensitization profile emerged (see the following paragraph 3.1 for a definition of sensitization). It should be noted that an age-related difference appeared in this profile (data not shown), where adult subjects with a treatment history of AMPH 10 showed significantly lower levels of hyperactivity compared to the corresponding periadolescent group. Accordingly, concomitant observational data indicated that this group of adult mice engaged particularly in AMPH-induced *compulsive licking* stereotypy. The general profile can be interpreted in the context of a response competition model. On the contrary, AMPH-10 treated Periadolescents showed a greater sensitization of the locomotor response, but failed to show the AMPH-induced stereotyped behavior that was typical of adults. Accordingly, subjects of this age were found not to develop a conditioned aversion for the pretreatment-paired environment (see Fig. 3, lower panels).

Overall, these results indirectly suggest that periadolescents may be more "protected" from AMPH-related aversive properties, and perhaps more vulnerable to the experience of internal states of reward, than are older animals. Thus, the present animal model of adolescence seems to represent a reliable and useful model for the investigation of the issue of vulnerability to a variety of habit-forming agents or emotional experiences whose positive reinforcing properties may rely upon a common neurobiological mechanism.

In the course of the same study, important age-related differences were also detected by the application, on observational data from the Testing day, of a multivariate statistical methodology (Principal Components Analysis, for literature and application see Ref. [46]) allowing each individual to be represented by co-ordinates in multidimensional space. As shown in Fig. 4, periadolescent baseline behavior (SAL-injected) was unbalanced towards self-directed activities, as animals of this group clustered closer to the *grooming* pole, whereas the corresponding adult group occupied an intermediate position. Upon acute AMPH administration, a significant shift from the pole of *grooming* to the opposite pole of *crossing–rearing* (environment-directed behaviors) was seen. Again, the shift exhibited by the young subjects was much more pronounced than that shown by adults. It seems therefore that periadolescent mice express in both cases a more unbalanced and "extreme-oriented" behavior than adults, and this result provides additional evidence that periadolescence represents a peculiar age-period from both behavioral and pharmacological point of view (for human studies, see also Ref. [1]).

## 3. Age-related changes in response to chronic or acute drug administration

### *3.1. Development of sensitization*

Substance abuse is a major issue in today's society and is of critical importance in the adolescent population. Research indicates that substance use is often initiated during the adolescent period and that brain reward areas are still undergoing changes during this time [10,46,71]. Despite this, only a few studies investigated the effects of chronic drug use on the reward mechanisms of periadolescent animals. The alterations that occur in these systems may produce, in reinforcement mechanisms, changes which make continued drug use more likely.

Repeated drug injections often result in a reduction in the magnitude of the drug's effects upon subsequent administration. This phenomenon, characterized by a shift to the

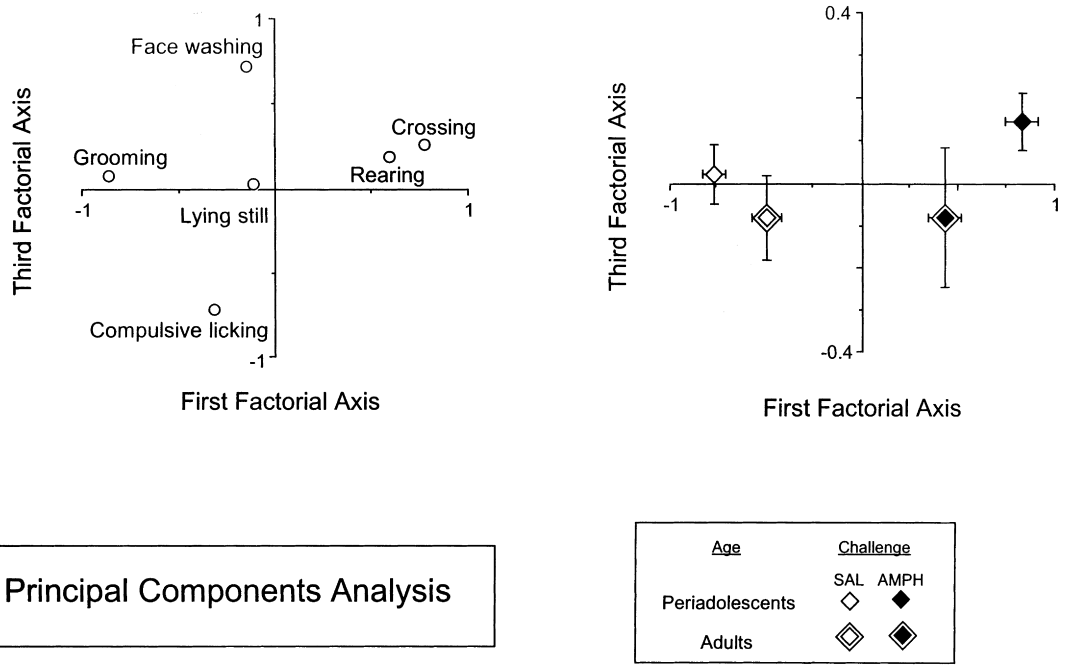


Fig. 4. Factorial axis ("*grooming* vs *crossing* and *rearing*"). Left panel: co-ordinates of the six behaviors considered. Right panel: mean ( ± SEM) co-ordinates of individuals as a function of type of challenge administered on Testing day and age ($n = 30$). (Reprinted with permission from Ref. [46].)

right in the dose-response curve, is known as tolerance. Conversely, some regimens of drug administration produce an increased response to the drug with subsequent administration. This phenomenon is associated with a shift to the left in the dose–response curve and is known as "reverse tolerance" or sensitization [79]. The development of both tolerance and sensitization to drug effects after repeated administration of the same agent is thought to contribute to the establishment of drug dependence and addiction both in animals and humans [80].

There is also ample evidence for interindividual variability in response to psychoactive drugs due to genetic, experiential or age-related causal factors [65,66,81–85]. For instance, young human subjects around or shortly after puberty report negligible effects after "snorting some lines" of cocaine, so that they may feel encouraged to do more to see what would happen [86]. Yet, the progression of cocaine use appears to be more rapid among human adolescents than among adult cocaine abusers, a finding which suggests that cocaine may have a greater addictive potential among adolescents than among adults [87]. This is of particular concern given that it is during adolescence that human subjects usually make the first contact with the drug, whose usage peaks in the early 20s [88].

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 (see Refs. [71,89,90]). The results summarized above indicate that, following chronic repeated and intermittent administration of high dosages of AMPH, a more marked sensitization of the locomotor response is typical of periadolescent subjects when compared to adults. Conversely, the former subjects failed to develop sensitization for the stereotyped pattern of behavior. Both these findings are strongly consistent with data from a very recent rat study [71], showing age-related differences in cocaine sensitization profile.

### *3.2. Assessment of sensitization to cocaine*

In this study, periadolescent (pnd 34–39) and adult (pnd 60–70) rats of both sexes were repeatedly administered cocaine (COC) for four consecutive days (see the legend of Fig. 5). Forty-eight hours after the last injection, all the animals were challenged with a standard 10 mg/kg cocaine dose, and their behavior was scored. As expected, acute cocaine induced a prominent increase in a number of behaviors, and this response profile was less marked in periadolescent compared to adult animals. The development of behavioral sensitization to cocaine compared to the chronic saline group (see differences in bar length between the white bars (the group receiving COC for the first time) and the shaded bars (the groups with a COC treatment history)) was a function of age-specific alterations in sensitivity to psychostimulants, with periadolescent animals showing sensitization to the locomotor activating effects of cocaine (see Fig. 5, upper panels). In contrast, no evidence of sensitized hyperactivity, but rather a consistent sensitization profile for both stereotyped *head scanning* and *focussed sniffing* activities was found in adults. The latter behavior

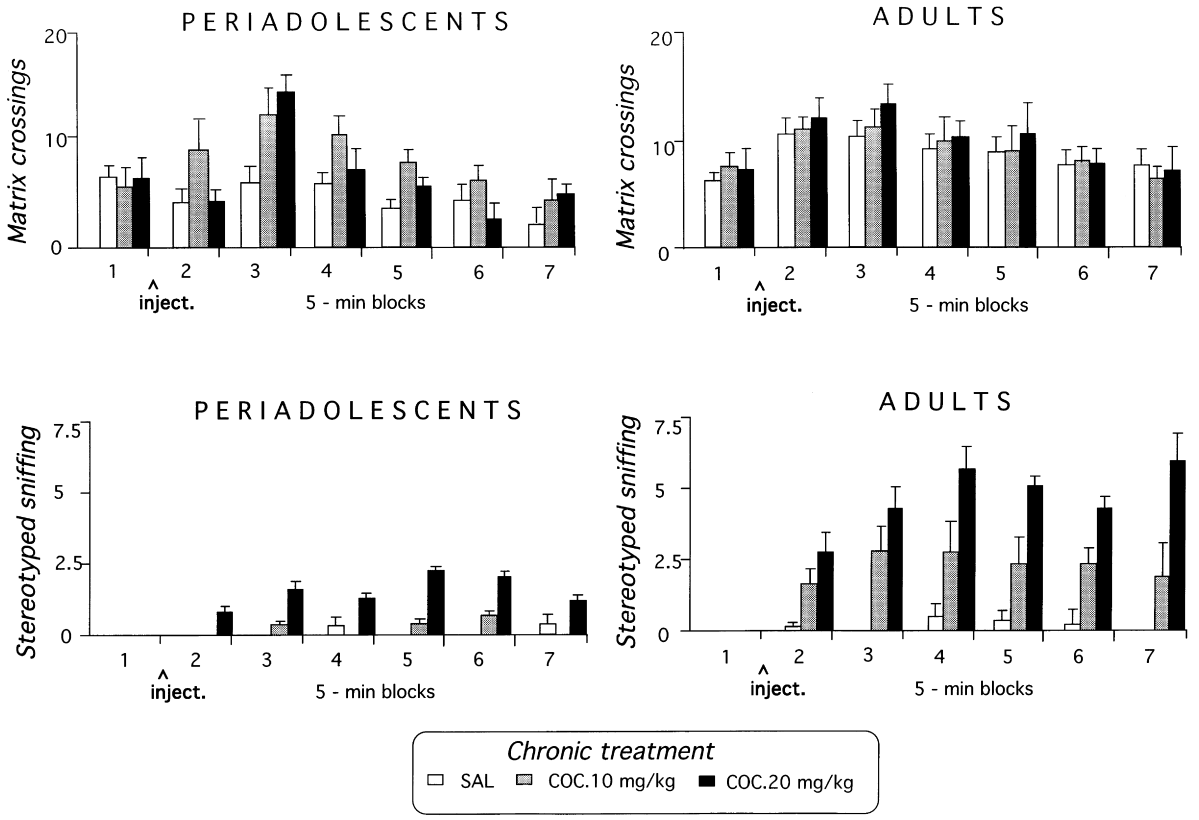


Fig. 5. Upper panels: Mean number of matrix crossings ( ± SEM) of periadolescent or adult female rats injected with a standard cocaine (10 mg/kg) dose on the Testing day. White bars indicate the acute response to cocaine by control animals (Sal Chronic treatment), whereas the shaded bars indicate animals repeatedly administered cocaine (Coc Chronic treatment, 10 or 20 mg/kg once/day) for four days in the test-chamber. Lower panels: mean frequency ( ± SEM) of stereotyped sniffing behavior by periadolescent or adult female rats. ($N = 8$–10 in each final group; animals are the same of the upper panels). (Reprinted with permission from Ref. [71].)

was found to be poorly expressed in periadolescents (see Fig. 5, lower panels). The progressive augmentation of stereotyped patterns of behavior also appeared to be more rapid among adult animals than among periadolescent animals. Such findings suggest that, as a function of age, distinct components of the behavioral repertoire are affected by repeated intermittent administration of the same drug doses. It is tempting to speculate that the lower frequency of drug-induced stereotypies among adolescent vs adult rats may also be seen as an index of differential affective components of the drug experience for animals of the two ages (see also Refs. [66,78,91] for discussion).

The expression of an augmented behavioral response to a challenge dose of a psychostimulant has been associated with enhanced DA release. Several studies have demonstrated that when exposed to psychostimulants for extended durations, biochemical and functional changes in DA systems are observed [92,93]. For instance, repeated stimulation-induced subsensensitive DA autoreceptors have been postulated to enhance DA release from drug-stimulated mesolimbic and nigrostriatal neuronal terminals and these changes could play a role in the behavioral sensitization phenomenon [93]. 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. Locomotion is elicited by drug-induced stimulation of dopaminergic mesolimbic brain areas, while stereotyped behaviors are associated with an increased dopaminergic activity at the extrapyramidal level [74]. There are reports that nigrostriatal DA neurons from immature rats are less sensitive to the inhibitory effects of cumulative amphetamine doses than neurons from adult rats [94]. This insensitivity might limit adaptations of stereotyped behavior, while changes might occur more easily in mesolimbic projections. Alternatively, it can be hypothesized that the nigrostriatal feedback pathways and/or the dopamine transporter in periadolescents may differ from those of adult rats (see Ref. [10]). The neurochemical mechanism for the decreased sensitivity to psychostimulants in periadolescent animals is unclear, as most indices of dopaminergic function including cell firing, terminal density dopamine content and transporter density and receptor number have achieved adult levels by this time [95]. However, some neuropharmacological reports suggest a differential degree of functional maturation for dopamine autoreceptors in mesolimbic and

striatal regions during the periadolescent period [96]. This developmental phenomenon has been hypothesized to account at least partially for the differences in the pattern of psychopharmacological sensitivity between animals of the two ages (see Ref. [11] for a review).

In the present study, chronic cocaine also reduced body weight and food consumption, but these alterations were evidenced in adult males only, and were not found in periadolescents of either sex. These profiles of chronic drug action suggest the involvement of a hormonal component; namely, the presence of high levels of testosterone, which makes adult males more susceptible than either younger and sexually immature animals or adult females. There is some evidence in the literature of an inverse relationship between plasma testosterone levels and the acute effects of amphetamine or cocaine, as well as between testosterone and the behavioral sensitization observed after repeated treatment with these drugs [97–100] (see also Refs. [71,101]).

## 4. Interplay between the response to novelty, the stress-response system and psychostimulants

### *4.1. Novelty, HPA and AMPH effects*

As discussed above, sensation seekers are often involved in risky and stressful activities that are avoided by others, as well as in illicit drug use [15]. The continuing search for novel stimuli may account in these subjects for the elevated levels of restlessness, recklessness, curiosity, and perhaps the first approach towards psychoactive drugs use, that appear to be widespread among human adolescents [6] (see also Ref. [1]). These data lead to the hypothesis that the experience associated with risk and novelty, as well as with psychostimulant drug use, may have a greater incentive potential among adolescents than among adult subjects (for discussion, see Ref. [46]).

In this context, a great interest has been recently devoted to the interaction between the brain reward system, i.e. the mesolimbic dopamine pathways, and the hormonal stress-response system, namely the hypothalamic–pituitary–adrenal (HPA) axis. The mesolimbic dopaminergic system has been shown to have a stimulatory action upon the HPA axis (see Refs. [102,103]). Indeed, a 6-OHDA lesion of dopaminergic neurons in the ventral tegmental area (VTA) causes a decrement in both basal and stress-induced CORT release [104]. It has been shown that the functional state of the HPA axis can modulate the rewarding effects of psychostimulant drugs (see Refs. [105,106] for a review; [107], see also Ref. [108]), and that the hormone corticosterone (CORT) has rewarding effects in itself, as it is readily self-administered by animals [109]. Also, a dysregulation in the negative feedback of stress-induced CORT secretion has been associated with a particular behavioral trait, consisting of elevated levels of novelty-seeking, increased behavioral reactivity to novelty, and individual vulnerability towards drug-taking behavior [4,107].

Furthermore, besides classical behavioral effects, psychostimulants (such as AMPH) activate the HPA axis and increase levels of both plasma adrenocorticotropic hormone (ACTH) and CORT [110]. Age-related differences in the hormonal effects of AMPH were thus hypothesized. So far, the experimental evidence pertaining (in animal models) to the interplay between a number of factors, such as the response to novelty and the function of the stress response system, as well as the effects of psychostimulant agents thereon, has been mainly derived from adult subjects (see Ref. [63]). Thus, in keeping with epidemiological data, it seemed of interest to investigate the issue of these psychobiological risk factors in an animal model of adolescence (see also Ref. [3]).

### *4.2. Forced exposure to novelty*

In an effort to characterize the animals' reaction to novelty, two different experimental paradigms are reported in the literature (see Refs. [4,63,111]). In the free-choice paradigm, which was adopted in the study discussed above, subjects can freely move between familiar and novel chambers within the apparatus. In this case, mice usually exhibit a marked preference for the novel environment [44,45,67,68] (see also Ref. [112]), and they fail to show any significant increase of blood CORT levels [113]. This indicates the absence of any stress-induced HPA axis activation. Conversely, in the forced-exposure paradigm, experimental subjects are placed into an inescapable novel environment. In these conditions, rats and mice show an integrated stress response, namely, a behavioral hyperactivity profile—consisting of both escape attempts and exploration [43,114,115]—and a prompt elevation in blood CORT levels [113,116,117].

As reported above, periadolescent mice were found to exhibit elevated levels of novelty-seeking in the free-choice paradigm compared to adults [46]. In that study, such behavioral differences among subjects of different ages were hypothesized to be linked to a peculiar function of the HPA axis. Thus, it was of interest to extend this characterization to the locomotor and hormonal profile emerging in a forced-novelty experimental paradigm (see below for the results of this study). In this setting, the integrated stress response was investigated by performing a detailed time-course analysis of both responses in mice of the two ages. Indeed, age-related discontinuities in response to psychological stress have been indicated as psychobiological risk factors for vulnerability to drugs of abuse [46,71,118].

### *4.3. Acute AMPH effects on locomotor activity*

A first experiment [9], characterized the acute behavioral effects of AMPH in both periadolescent and adult mice when faced with an inescapable and novel environment. With respect to locomotion, the results indicated (see

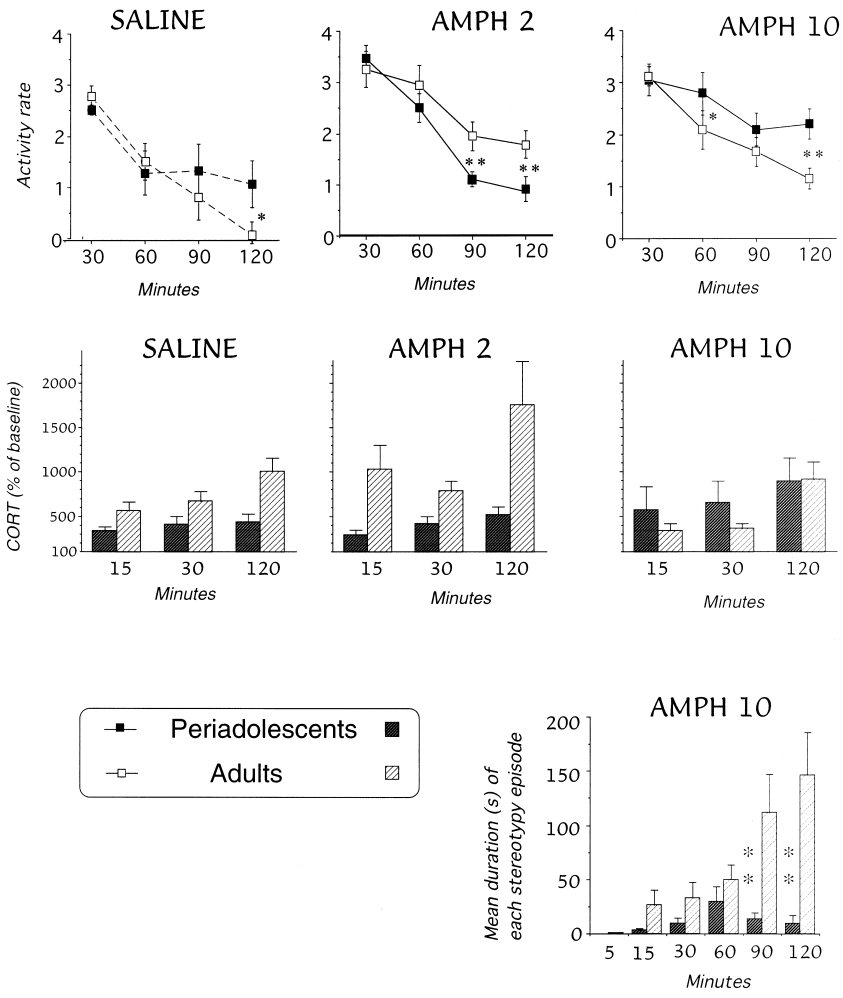


Fig. 6. Upper panels: mean ( ± SEM) activity (number of photobeam interruptions/s). Animals of both ages were injected with either SAL or AMPH (2 or 10 mg/kg) immediately before being placed in the novel environment. *$p < 0.05$, **$p < 0.01$, in multiple comparisons performed between the two ages within the same treatment. Middle panels: mean ( ± SEM) plasma corticosterone levels shown by subjects of both ages, measured as percentage of increase over the baseline. Animals were the same as above. At specific time points, animals were sacrificed and trunk blood collected. Lower panel: mean ( ± SEM) duration of each single episode of *Lick–Gnaw* behavior (total duration/frequency). Animals were the same as above. **$p < 0.01$ in multiple comparisons performed between the two ages. ($N = 10$). (Reprinted with permission from Ref. [9].)

Fig. 6, upper panels) that when compared to adults, periadolescent SAL-injected controls were characterized by activity levels that were still elevated at the end of a long-lasting 120-min session. These results might be interpreted as an evidence of a deficit in habituation to novel stimuli in the case of periadolescent subjects. Consistent with this proposal, previous reports showed that periadolescent rats and mice are characterized by elevated basal levels of behavioral activation and that they are apparently somewhat unable to focus their attention on salient environmental cues (for a review, see Ref. [11]).

As expected, the administration of an AMPH 2 dosage produced the well-known increase in locomotor and exploratory activity. However, a lower hyperactivity profile was shown by periadolescents when compared to adults. This is consistent with previous reports, showing that animals around this developmental stage show a characteristic hyporesponsivity to the locomotor effects of acute administration of catecholamine agonists such as cocaine and amphetamine, and an accentuated behavioral response to a catecholamine antagonist such as haloperidol (see also Refs. [10,11,46,71,119]). For the behavioral profile of AMPH 10-treated subjects of both ages, see Section 4.5.

As outlined in a classical review by Spear and Brake [11], when compared with younger or older animals, periadolescent rats show alterations in psychopharmacological sensitivity, which apparently do not rely on an age-specific decrease in brain drug availability, but rather appear to be related to an alteration in nervous system sensitivity. To test this hypothesis, Spear and Dendel (cited in Ref. [11]) examined brain amphetamine levels in rats of both sexes at various ages after injection of 5 mg/kg AMPH. At all time periods after injection, brain AMPH levels did not differ among pnd 35, pnd 45 and adults. However, the behavioral response pattern to AMPH was similar in young and adult rats; it was the 35-day-old rats that exhibited the relatively

attenuated response to AMPH. These results agree with a report by Campbell and colleagues [119], in which age-related decreases in drug sensitivity were found to be substantially independent from the route of drug administration (PO, IP, ICV), which was systematically varied in an attempt to bypass potential "pharmacokinetic" influences.

A number of developmental factors may be involved in this phenomenon, one of which might be a temporary decrease in the functional efficacy of mesolimbic dopamine projections resulting in decreased overall activity of the dopaminergic system (see Ref. [11]). Importantly, recent studies reported lower dopamine levels in periadolescent rats [120,121], and this lower dopamine level is consistent with the hypothesis of a lower functional activity within the mesolimbic system. Indeed, the behavioral and pharmacological profile shown by periadolescent rodents seems to resemble that of the adult animals with lesions of the dopaminergic system (see Ref. [11]). Also, the functional development of dopamine autoreceptors, which actually seem to reach a functional maturation during periadolescence, has been suggested [11] as a possible causal factor. These authors proposed that the onset of this negative-feedback control might be responsible for a transient hyporesponsivity within the mesolimbic system. Indeed, some dopaminergic agonists are able to reduce spontaneous locomotor activity, possibly via activation of the D2 autoreceptor, and administration of a low dose of apomorphine reduces spontaneous activity in 35-day-old rats, but not in younger ones [122].

However, other findings are apparently in contrast with this picture. In microdialysis studies, the administration of dopaminergic agonists, such as quinpirole or apomorphine, are reported to decrease dopamine release as early as 5 days of age [120,123]. These studies support the idea that presynaptic autoreceptors are already functional in early infancy. Thus, maturation of dopamine autoreceptors seems to be achieved after weaning, and possibly during periadolescence, when considering a behavioral parameter such as locomotor activity, but not when considering a neurochemical parameter, such as dopamine release.

To account for the paradoxical behavior and neurochemical findings in periadolescent rats, an additional and more recent hypothesis implicates a mechanism involving a transient elevation in DA transmission. The latter can have a role in opposing the dopaminergic–cholinergic relationship which has been proposed with respect to the regulation of brain structures that control motor function [124–126]. The decreased cholinergic tone is an expected secondary consequence resulting from increased dopaminergic tone [126,127]. This results in post-synaptic supersensitivity of cholinergic receptors and consequently increased cholinergic tone, which may mediate behavioral subsensitivity when challenged with dopaminergic drugs [10]. The authors propose that, in periadolescent subjects, a more efficient regulation of cholinergic neurons by DA leads to a transient up-regulation of post-synaptic striatal cholinergic receptors. Hence, behavioral subsensitivity during periadolescence could be attributed to increased cholinergic transmission despite an increased regulatory influence of DA on striatal cholinergic interneurons. Considering this view, an upregulation of postsynaptic dopamine receptors in rat striatal slices has been suggested to be typical of periadolescence [10].

### *4.4. Acute AMPH effects on corticosterone release*

In the course of the study reviewed above, important age-related differences were also found in the hormonal assessment, with naive non-injected periadolescent mice exhibiting two-fold basal plasma CORT levels compared to adults ($10.5 \pm 4.5$ vs $4.7 \pm 1.9$ μg/ml, respectively). This may suggest a higher level functionality in the neuroendocrine HPA axis of animals around this age (see also Ref. [128]), which may be ascribed, at least for males, to an immaturity in gonadal function. Indeed, a chronic inhibitory androgen-mediated action on baseline HPA activity has been hypothesized [98,99], and peripubertal rodents are known to have a much lower testicular weight/body weight ratio than adults [129].

As shown in Fig. 6 (middle panels), following a forced exposure to novelty, adult SAL-injected subjects exhibited an increasing profile of CORT release as the session progressed, whereas only a tendency towards a slight elevation was found for periadolescents. These results indicate periadolescence to be associated in mice with a reduced neuroendocrine response to a prolonged condition of mild psychological stress. Thus, the HPA axis seems to be somewhat hyporesponsive to external perturbations during this developmental stage. Interestingly, a stress-hyporesponsive period—taking place during the first two postnatal weeks—has been reported in infant rats and mice (see Ref. [118] for a review, see also Ref. [130]]. However, apparently contrasting results have also been reported, with an inverted profile of age-related differences appearing in the case of repeated and intermittent stress exposure. Greater behavioral effects of stress are found in pre-pubertal mice, as compared to mature ones [131]. As expected on the basis of the literature [110,132], the administration of the AMPH 2 dose also produced a prominent increase of CORT release in adult subjects, when compared to SAL-injected controls. In animals of this age, the elevated AMPH 10 dose actually seemed to reduce blood CORT concentrations, suggesting that an inverted-U-shaped profile could account for this kind of dose–response curve. For a possible role of behavioral stereotypies, see Section 4.5.

With respect to the periadolescent group, in the absence of AMPH 2-induced changes, only a tendency towards increased CORT levels was found with the administration of the high AMPH 10 dosage. Thus, a shift to the right in the dose–response curve for the AMPH-induced CORT release (measured as percent of the baseline) is suggested during periadolescence, apparently resulting also in a marked

hyporesponsivity of the HPA axis to AMPH administration. Such a finding is particularly intriguing in view of the few studies devoted to the ontogenetic characterization of AMPH-induced CORT release. A recent study actually noted an AMPH-induced reduction in CORT levels in 18-day-old animals treated with an AMPH 3 dosage [118], whereas the opposite profile is typical of adults (present data; [110,132]). Thus, from these data, periadolescence appears to have the characteristics of an age of transition for the function of the HPA axis.

As the function of the mesolimbic dopamine system is known to modulate HPA axis activity [102–104], a number of hypotheses can be formulated. Firstly, the neuronal pathways by which the latter is controlled by the former may be not yet mature; secondly, the mesolimbic dopamine pathways may undergo a reduced stimulation by AMPH during periadolescence. In this view, however, it has been shown that DA receptors modulating CORT secretion are already fully mature in 30-day-old rats [133]. Further work would be necessary to investigate developmental changes in the control of the HPA response by brain systems targeted by AMPH administration.

In this framework, a series of very recent studies by Kellogg's group [134,135] have pointed to the role played by maturational changes occurring in stressor-sensitive forebrain dopamine projections and related neural systems during the adolescent period. Thus, the noradrenergic (NE) projection from the brainstem to the hypothalamus, which has been shown to influence an organism's ability to appropriately react or adapt to an episode of environmental challenge, seems not to have achieved an adult state by late juvenile ages but continues to undergo changes in function throughout adolescence.

In these studies, the effect of an environmental stressor, such as restraint, markedly reduced the release of hypothalamic NE at the juvenile (pnd 28), had no effect at the adolescent (pnd 42) and slightly, but significantly, increased release in the adult (pnd 70) male rat. While the hormonal (CORT) response to the same stressor was similar across the ages studied, the hypothalamic NE response to a stressor varied across adolescent development. Thus, while adults are reported to meet a challenge by increasing NE utilization, juveniles appear to meet the same challenge by decreasing utilization. Juveniles and adolescents appear to respond to a mild environmental challenge by conserving and limiting the use of their endogenous transmitter stores. The stressor-responsiveness of this system appears to be blunted at mid-adolescence. We should therefore consider that the development of this catecholaminergic system may play a key role in the emergence of appropriate adult neural and behavioral responses to stressful or challenging situations. As outlined by Kellogg [134,135], as changing systems are vulnerable to perturbation, the changes taking place in the hypothalamus during adolescence may contribute not only to the emergence of adult-typical responses but also to the appearance of clinical disorders during adolescence.

### *4.5. Acute effects of amphetamine administration on stereotyped patterns of behavior*

In keeping with the results of the studies reported above [46,66], a fine-grain behavioral analysis of the AMPH-induced stereotypy profile was carried out (for the ontogeny of spontaneous stereotypies, see Ref. [136]; for the ontogeny of AMPH-induced stereotypies, see Ref. [66]). In response to acute administration of a high drug dosage, AMPH 10 treated periadolescents were more involved in elevated levels of locomotion across the whole session (see Fig. 6, upper panels) compared with the corresponding Adult group. Conversely, when compared to periadolescents, adult subjects exhibited almost maximal levels of the compulsive *Lick–Gnaw* stereotypy (see Fig. 6, bottom panel). This general profile can be interpreted in the context of a response competition model. In these animals, the stereotyped behavioral profile was particularly interesting, as each single compulsive episode was very intense and lasted several minutes. Such stereotyped activity also appeared to be "focused", as adult subjects scarcely moved away from the part of the floor to which they were compulsively directing their attention.

Stereotyped behavioral syndromes are thought to rely on AMPH-induced stimulation of the dopamine pathways in the *caudato-putamen* area in the CNS [73,74]. The present behavioral results may be compared with a recent characterization of developmental profile of dopamine receptors in the rat brain. An overexpression of striatal DA receptors is reported to occur prior to puberty (pnd 40), receptor density decreasing to adult levels thereafter [137]. 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 the adults. As outlined by Teicher and associates [138], these and related findings support the hypothesis that the marked overproduction and elimination of synapses and receptors during adolescence may serve as a permissive factor for a number of psychiatric disorders, including schizophrenia, attention-deficit hyperactivity disorder, and perhaps substance abuse. 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 [139].

As mentioned above, sterotypies have been proposed to serve as a behavioral mechanism for coping with drug-induced excessive arousal [76,77]. The present results in adult mice are in line with this hypothesis, as AMPH 10-treated subjects showed at the same time a very intense stereotyped behavioral syndrome and a reduction of AMPH-induced CORT levels. However, the literature on this issue is mixed, and contrasting results are also available (see Refs. [110,118,140]); further work seems essential. Nevertheless, it is noteworthy that such an integrated behavioral and neuroendocrine response is apparently absent in mice around the periadolescent period.

In animal models, a dysregulation of CORT secretion has been identified as a crucial factor in individual susceptibility towards risk, as well as drug-taking behaviors (see also Ref. [4] for a review). Indeed, involvement in risky activities is usually associated at least to a certain degree with the experience of psychological stress, which ultimately leads to a prompt activation of neuroendocrine pathways in the HPA axis and an increase in plasma CORT levels [141]. This hormone has actually been shown in rats to have intrinsic rewarding properties, and to potentiate the incentive effects of psychostimulants that are abused by humans (see above). As such, it was hypothesized that periadolescent and adult mice would exhibit substantial differences in their behavioral and hormonal response to a mild psychological stress, such as forced exposure to a novel environment.

To summarize, we have shown that periadolescent mice: (1) are characterized by a higher basal function of the hormonal stress-response system; (2) exhibit a reduced habituation to the novel environment, which might perhaps account for their elevated basal levels of locomotion; and (3) show a lower integrated behavioral and physiological response to a mild stress condition. In agreement with previous findings, reduced response to an acute AMPH administration was seen in animals during periadolescence. Similarly, periadolescents have been shown to exhibit a reduced sensitivity to AMPH-induced place conditioning [46], as well as a marked resistance to AMPH-induced taste aversion [142]. When considered as a whole, these data consistently support a picture of hyporesponsivity to the acute effects of psychostimulants in subjects around this age, from a behavioral, hormonal and motivational point of view (for a review, see Ref. [11]). This complex and integrated profile has also been associated with age-related discontinuities in mesolimbic and striatal dopaminergic systems [137].

On the basis of these considerations, it can initially be concluded that periadolescents are somehow "protected" from the acute effects of psychostimulant agents, and a certain degree of "invulnerability" towards the addictive risk of these drugs might be expected. However, as outlined by Ramsay and Woods [143], the initial insensitivity to experience psychoactive drugs might underlie an increased risk to subsequently develop addiction. This might be particularly true among human adolescents, since initially insensitive individuals are perhaps more likely to have repeated experiences with a drug (due to its low impact among them), and hence to develop problems linked with drug abuse. On this view, it is interesting to note the anedoctal report that young human subjects around or shortly after puberty report negligible effects after "snorting some lines of cocaine" [86], yet the progression of cocaine use appears to be more rapid among this group than among adults [87]. As for animal models, following repeated and intermittent administration of either cocaine or AMPH, a marked sensitization of the locomotor response is typical of periadolescent subjects [46,71]. As the same neural area within the CNS (namely, the *nucleus accumbens*) is implicated both in AMPH-induced locomotor hyperactivity and in the modulation of AMPH-induced reward (see Refs. [42,75]), 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.

## 5. Concluding remarks

During the period of late childhood and adolescence, neurobiological systems are still undergoing important developmental rearrangements. Early in infancy, final brain size and the number of available neurons and axons appear to be established. However, plasticity of the brain continues during adolescence through an integrated process of overproduction and elimination of synapses, evolution of neurotransmitter systems, and progressive myelination (for literature and discussion, see Ref. [3]). In addition, hormonal levels change dramatically during adolescence as a result of the onset of puberty. Concomitant to these changes in brain and hormonal status, significant transitions occur in cognitive, psychological, and social competence. However, as outlined by Witt [3], the potential impact of environmental factors during adolescence, including psychoactive agents consumption, has received surprisingly little investigation. Yet, these factors may have a strong impact on the unique neurobiological and psycho-physiological strengths and weaknesses that predispose or protect an individual 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.

As the results of the studies illustrated above indicate, when tested in a free-choice novelty preference paradigm, periadolescent mice are more aroused and spend a significantly higher percentage of time in a novel compartment, than the corresponding adult group [46]. These results suggest that, across different mammalian species, adolescence emerges as a period characterized by a strong inner drive to search for novel stimuli. Such a novelty-seeking trait, which has been shown to be typical of human adolescents [14], may perhaps explain the first approaches towards the experience of psychoactive agents. Moreover, it seems that in animal models the search for novel experiences activates the brain's reward system in the same way as do drugs of abuse (for a review, see Ref. [63]). The fact that novelty-seekers may be tapping into the same primal reward mechanisms as drug abusers provide a biological interpretation of the fact that individuals who constantly seek new and exciting experiences are much more likely to abuse drugs than are individuals who have less need for novel stimulation. This finding suggests new ways to reach such individuals with drug abuse prevention interventions.

It has often been assumed that altered social needs, peer pressure, and other socio-psychological variables have a strong influence on the emergence of behavioral anomalies seen in human adolescents. The contribution of alterations in brain anatomy and physiology to the behavioral characteristics of human adolescents has not seriously been considered. Yet, a deeper understanding of such factors appears to be worthy of inclusion in both social and health programmes.

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## References

[1] Compas BE, Hinden BR, Gerhardt CA. Adolescent development: pathways and processes of risk and resilience. Annu Rev Psychol 1995;46:265–93.
[2] Gerra G, Zaimovic A, Giucastro G, Maestri D, Monica C, Sartori R, caccavari R, Delsignore R. Serotonergic function after ±3,4-methylene-dioxymethamphetamine (ecstasy) in humans. Int Clin Psychopharmacology 1998;13:1–9.
[3] Witt ED. Mechanisms of alcohol abuse and alcoholism in adolescents: a case for developing animal models. Behav Neural Biol 1994;62:168–77.
[4] Dellu F, Piazza PV, Mayo W, Le Moal M, Simon H. Novelty-seeking in rats: biobehavioral characteristics and possible relationship with the sensation-seeking trait in man. Neuropsychobiology 1996;34:136–45.
[5] Laviola G, Terranova ML. The developmental psychobiology of behavioural plasticity in mice: the role of social experiences in the family unit. Neurosci Biobehav Rev 1998;23:197–213.
[6] Mathias R. Students' use of marijuana, other illicit drugs, and cigarettes continued to rise in 1995. NIDA Notes 1996;11:8–9.
[7] Yamaguchi K, Kandel DB. Patterns of drug use from adolescence to young adulthood. III. Predictors of progression. Am J Publ Health 1984;74:673–81.
[8] Newcomb MD. Identifying high-risk youth: prevalence and patterns of adolescent drug abuse. NIDA Res Mon Series 1985;156:7–37.
[9] Adriani W, Laviola G. A unique hormonal and behavioral hyporesponsivity to both forced novelty and *d*-amphetamine in periadolescent mice. Neuropharmacology 1999, in press.
[10] Bolanos CA, Glatt SJ, Jackson D. Subsensitivity to dopaminergic drugs in periadolescent rats: a behavioral and neurochemical analysis. Dev Brain Res 1998;111:25–33.
[11] Spear LP, Brake SC. Periadolescence: age-dependent behavior and psychopharmacological responsivity in rats. Dev Psychobiol 1983;16:83–109.
[12] Anthony JC, Petronis KR. Early-onset drug use and risk of later drug problems. Drug Alcohol Dep 1995;40:9–15.
[13] Breslau N, Peterson EL. Smoking cessation in young adults: age at initiation of cigarette smoking and other suspected influences. Am J Pub Health 1996;86:214–20.
[14] Zuckerman M. Behavioral expressions and biosocial bases of sensation seeking, Cambridge, MA: Cambridge University Press, 1994.
[15] Wills TA, Vaccaro D, McNamara G. Novelty seeking, risk taking and related constructs as predictors of adolescent substance use: an application of Cloninger's theory. J Subst Ab 1994;6:1–20.
[16] Arnett J. Reckless behavior in adolescence: a developmental perspective. Dev Rev 1992;12:339–73.
[17] Zuckerman M. The psychobiological model for impulsive unsocialized sensation seeking: a comparative approach. Neuropsychobiology 1996;34:125–9.
[18] Ebstein RP, Novick O, Umansky R, Priel B, Osher Y, Blaine D, Bennet ER, Nemanov L, Katz M, Belmaher RH. Dopamine D4 receptor (D4DR) exon III polymorphism associated with the human personality trait of novelty seeking. Nature Genet 1996;12:78–80.
[19] Netter P, Hennig J, Roed IS. Serotonin and dopamine as mediators of sensation seeking behavior. Neuropsychobiology 1996;34:155–65.
[20] McCann UD, Ridenour A, Shaham Y, Ricaurte GA. Serotonin neurotoxicity after ±3,4-methylene-dioxymethamphetamine (MDMA, ecstasy): a controlled study in humans. Neuropsychopharmacology 1994;10:129–38.
[21] Randall T. Ecstasy-fueled rave parties become dances of death for English youths. Journal of the American Medical Association 1992;269:1505–6.
[22] Ricaurte GA, DeLanney LE, Irwin I, Langston JW. Toxic effects of MDMA on central serotonergic neurons in the primate: importance of route and frequency of drug administration. Brain Res 1988;446:165–8.
[23] Schmidt CJ. Neurotoxicity of the psychedelic amphetamine, methylenedioxymethamphetamine. J Pharmacol Exp Ther 1987;240:1–7.
[24] Brodkinn J, Malyala A, Nash JF. Effect of acute monoamine depletion on 3,4-methylene-dioxymethamphetamine-induced neurotoxicity. Pharmacol Biochem Behav 1993;45:647–53.
[25] Cadet JL, Ladenheim B, Hirata H, Rothman RB, Ali S, Carlson E. Superoxide radicals mediate the biochemical effects of methylenedioxymethamphetamine (MDMA): evidence from using CUZn-superxide dismutase transgenic mice. Synapse 1995;21:169–76.
[26] McCann UD, Ricaurte GA. Reinforcing subjective effects of ±3,4-methylene-dioxymethamphetamine (ecstasy) may be separable from its neurotoxic actions: clinical evidence. J Clin Psychopharmacol 1993;13:214–7.
[27] Gerra G, Zaimovic A, Chittolini B, Giucastro G, Palladino M, Gaggiotti MT, Caccavari R. Methylene-dioxymethamphetamine: neuroendocrine and behavioural aspects (in italian). Quaderno di Psichiatria 1996;3:159–67.
[28] Green AR, Cross AJ, Goodwin GM. Review of the pharmacology and clinical pharmacology of 3,4-methylene-dioxymethamphetamine (MDMA or ecstasy). Psychopharmacology 1995;119:247–60.
[29] Greer G, Strassman RJ. Information on ecstasy (letter). Am J Psychiatry 1985;142:1391.
[30] Solowij N, Hall W, Lee N. Recreational MDMA use in Sydney: a profile of ecstasy users and their experiences with the drug. Br J Addiction 1992;87:1161–72.
[31] Katz MM, Maas JW, Frazer A, Koslow SH, Bowden CL, Berman N, et al. Drug-induced actions on brain neurotransmitter system and changes in the behaviors and emotions of depressed patients. Neuropsychpharmacology 1994;11:89–100.
[32] Young LT, Warsh JJ, Kish SJ, Shannak K, Hornykeiwicz O. Reduced brain 5-HT and elevated NE turnover and metabolites in bipolar affective disorder. Biol Psychiatry 1994;35:121–7.

[33] Jerison HJ. Evolutionary biology of intelligence: the nature of the problem. In: Jeriso HJ, Jerison I, editors. Intelligence and evolutionary biology, Berlin: Springer, 1988. p. 1–11.
[34] Renner MJ, Seltzer CP. Molar characteristics of exploratory and investigatory behavior in the rat (*Rattus norvegicus*). J Comp Psychol 1991;105:326–39.
[35] Wilz KJ, Bolton RL. Exploratory behavior in response to the spatial rearrangement of familiar stimuli. Psychon Sci 1971;24:117–8.
[36] Rebec GV, Christiansen JRC, Guerra C, Bardo MT. Phasic increases in extracellular dopamine during a free-choice novelty task as measured by fast-scan voltammetry. Soc Neurosci Abstr 1996;22:159.
[37] Fink JS, Smith GP. Mesolimbic and mesocortical dopaminergic neurons are necessary for normal exploratory behavior in rats. Neurosci Lett 1980;17:61–5.
[38] Pierce CR, Crawford CA, Nonneman AJ, Mattingly BA, Bardo MT. Effects of forbrain dopamine depletion on novelty-induced place preference behavior in rats. Pharmacol Biochem Behav 1990;36:321–5.
[39] Hoebel BG, Monaco AP, Hernandez L, Aulisi EF, Stanley BG, Lenard L. Self-injection of amphetamine directly into the brain. Psychopharmacology 1983;81:158–63.
[40] Robbins TW, Everitt BJ. Neurobehavioural mechanisms of reward and motivation. Curr Opin Neurobiol 1996;6:228–36.
[41] Roberts DC, Corcoran ME, Fibiger HC. On the role of ascending catecholaminergic systems in intravenous self-administration of cocaine. Pharmacology Biochemistry and Behavior 1977;6: 615–20.
[42] Wise RA. Neurobiology of addiction. Curr Opin Neurobiol 1996;6:243–51.
[43] Renner MJ. Neglected aspects of exploratory and investigatory behavior. Psychobiology 1990;18:16–22.
[44] Bardo MT, Neisewander JL, Pierce RC. Novelty-induced place preference behavior in rats: effect of opiate and dopaminergic drugs. Pharmacol Biochem Behav 1988;32:683–9.
[45] Misslin R, Ropartz P. Effects of metamphetamine on novelty-seeking behavior by mice. Psychopharmacology 1981;75:39–43.
[46] Adriani W, Chiarotti F, Laviola G. Elevated novelty seeking and typical *d*-amphetamine sensitization in periadolescent compared to adult mice. Behav Neurosci 1998;112:1152–66.
[47] Cirulli F, Terranova ML, Laviola G. Affiliation in periadolescent rats: behavioral and corticosterone response to social reunion with familiar and unfamiliar partners. Pharmacol Biochem Behav 1996;54:99–105.
[48] Meaney MJ, Stewart J. A descriptive study of social development in the rat (*Rattus norvegicus*). Animal Behav 1981;29:34–45.
[49] Panksepp J. The ontogeny of play in rats. Dev Psychobiol 1981;14:327–32.
[50] Terranova ML, Cirulli F, Laviola G. Behavioral and hormonal effects of partner familiarity in periadolescent rat pairs upon novelty exposure. Psychoneuroendocrinology 1999;24:639–56.
[51] Terranova ML, Laviola G, Alleva E. Ontogeny of amicable social behavior in the mouse: gender differences and ongoing isolation outcomes. Dev Psychobiol 1993;26:467–81.
[52] Terranova ML, Laviola G, DeAcetis L, Alleva E. A description of the ontogeny of mouse agonistic behavior. J Comp Psychol 1998;112:3–12.
[53] Thor DH, Holloway WR. Developmental analyses of social play behavior in juvenile rats. Bull Psychon Soc 1984;22:587–90.
[54] Williams E, Scott JP. The development of social behavior patterns in the mouse, in relation to natural periods. Behaviour 1953;6:35–65.
[55] Barkley MS, Goldman BD. A quantitative study of serum testosterone, sex accessory organ growth, and the development of intermale aggression in the house mouse. Horm Behav 1977;8: 208–18.
[56] Benton D, Brain PF. Behavioural comparison of isolated, dominant and subordinate mice. Behav Proc 1979;4:211–9.
[57] Bronson FH. Mammalian reproductive biology, Chicago: University of Chicago Press, 1989.
[58] McKinney TD, Desjardins C. Postnatal development of the testis and fighting behaviour and fertility in mice. Biological Reproduction 1973;9:279–94.
[59] Dixon AK, Fish HU, McAllister KH. Ethopharmacology: a biological approach to the study of drug-induced changes in behavior. Adv Study Behav 1990;19:171–83.
[60] Haber SB, Simmel EC. Tail rattling and agonistic behavior in mice: coincidental or causal?. Bull Psychon Soc 1976;7:84–6.
[61] Krsiak M. Tail rattling in aggressive mice as a measure of tranquillizing activity of drugs. Activ Nerv Sup 1975;17:225–6.
[62] Galef BG. The ecology of weaning: parasitism and the achievement of independence by altricial mammals. In: Gubernick DJ, Klopfer PH, editors. Parental care in mammals, New York: Plenum Press, 1981. p. 211–41.
[63] Bardo MT, Donohew RL, Harrington NG. Psychobiology of novelty seeking and drug seeking behavior. Behav Brain Res 1996;77:23–43.
[64] Benjamin J, Li L, Patterson C, Greenberg BD, Murphy DL, Hamer DH. Population and familial association between the D4 dopamine receptor gene and measures of novelty seeking. Nature Genet 1996;12:81–4.
[65] Laviola G, Dell'Omo G, Alleva E, Bignami G. Ontogeny of cocaine hyperactivity and conditioned place preference in mice. Psychopharmacology 1992;107:221–8.
[66] Laviola G, Dell'Omo G, Chiarotti F, Bignami G. *d*-Amphetamine conditioned place preference in developing mice: relation with changes in activity and stereotypies. Behav Neurosci 1994;108: 514–24.
[67] Laviola G, Adriani W. Evaluation of unconditioned novelty seeking and *d*-amphetamine conditioned motivation in mice. Pharmacol Biochem Behav 1998;59:1011–20.
[68] Hughes RN. Behavior of male and female rats with free choice of two environments differing in novelty. Animal Behav 1968;16:92–6.
[69] Glickman SE, Schiff BB. A biological theory of reinforcement. Psychol Rev 1967;74:81–109.
[70] Glick SD, Milloy S. Rate-dependent effects of *d*-amphetamine on locomotor activity in mice: possible relationship to paradoxical amphetamine sedation in minimal brain dysfunctions. Eur J Pharmacol 1973;24:266–8.
[71] Laviola G, Wood RD, Kuhn C, Francis RLP. Cocaine sensitization in periadolescent and adult rats. J Pharmacol Exp Ther 1995;275:345–57.
[72] Lett BT. Enhancement of conditioned preference for a place paired with amphetamine produced by blocking the association between place and amphetamine-induced sickness. Psychopharmacology 1988;95:390–4.
[73] Kelly PH, Seviour PW, Iversen S. Amphetamine and apomorphine responses in the rat following 6-OHDA lesions of the nucleus accumbens septi and corpus striatum. Brain Res 1975;94:507–22.
[74] Staton DM, Solomon PR. Microinjections of *d*-amphetamine into the nucleus accumbens and caudate-putamen differentially affect stereotipy and locomotion in the rat. Physiol Psychol 1994; 12:159–62.
[75] Wise RA, Bozarth MA. A psychomotor stimulant theory of addiction. Psychol Rev 1987;94:469–92.
[76] Jones GJ, Mittleman G, Robbins TW. Attenuation of amphetamine-stereotypy by mesostriatal dopamine depletion enhances plasma corticosterone: implications for stereotypy as a coping response. Behav Neural Biol 1989;51:80–91.
[77] Mittleman G, Jones GH, Robbins TW. Sensitization of amphetamine-stereotypy reduces plasma corticosterone: implications for stereotypy as a coping response. Behav Neural Biol 1991;56:170–82.
[78] Wall A, Hinson RE, Schmidt E, Johnson C, Streather A. Place

conditioning with *d*-amphetamine: the effect of the CS-UCS interval and evidence of a place avoidance. Anim Learn Behav 1990;18:393–400.
[79] Stewart J, Badiani A. Tolerance and sensitization to the behavioral effects of drugs. Behav Pharmacol 1993;4:289–312.
[80] Goudie AJ, Emmett-Oglesby MW. Psychoactive drugs: tolerance and sensitization, New Jersey: Humana Press, 1989.
[81] Deminiere JM, Piazza PV, LeMoal M, Simon H. Experimental approach to individual vulnerability to psychostimulant addiction. Neurosci Biobehav Rev 1989;13:141–7.
[82] Koff JM, Shuster L, Miller LG. Chronic cocaine administration is associated with behavioral sensitization and time-dependent changes in striatal dopamine transporter binding. J Pharmacol Exper Ther 1994;268:277–82.
[83] Laviola G, Dell'Omo G. Precocious weaning and changes in social variables during pre-puberty affect cocaine reinforcing properties in adult mice. Psychobiology 1997;25:163–70.
[84] Laviola G, Renna G, Bignami G, Cuomo V. Ontogenetic and pharmacological dissociation of various components of locomotor activity and habituation in the rat. Int J Dev Neurosci 1988;6:431–8.
[85] Spear LP, Enters EK, Linville DG. Age-specific behaviors as tools for examining teratogen-induced neural alterations. Neurobehav Toxicol Teratol 1985;7:691–5.
[86] Weiss RD, Mirin SM, Bartel RL. Cocaine, Washington, DC: American Psychiatric Press, 1994.
[87] Estroff TW, Schwartz RH, Hoffman NG. Adolescent cocaine abuse: addictive potential, behavioral and psychiatric effects. Clin Pediatr 1989;28:550–5.
[88] Johanson C, Fishman MW. The pharmacology of cocaine related to its abuse. Pharmacol Rev 1989;41:3–52.
[89] McDougall SA, Duke MA, Bolanos CA, Crawford CA. Ontogeny of behavioral sensitization in the rat. Effects of direct and indirect dopamine agonists. Psychopharmacology 1994;116:483–90.
[90] Wood RD, Tirelli E, Snyder KJ, Heyser CJ, LaRocca TM, Spear LP. Evidence for behavioral sensitization to cocaine in preweanling rat pups. Psychopharmacology 1998;138:114–23.
[91] Mason GJ. Stereotypies: a critical review. Anim Behav 1991;41:1015–37.
[92] Kalivas PW, Sorg BA, Hooks MS. The pharmacology and neural circuitry of sensitization to psychostimulants. Behav Pharmacol 1993;4:315–34.
[93] Yi S, Johnson KM. Chronic cocaine treatment impairs the regulation of synaptosomal $^{3}$H-DA release by D2 autoreceptors. Pharmacol Biochem Behav 1990;36:457–61.
[94] Trent F, Nakamura S, Tepper JM. Amphetamine exerts anomalous effects on dopaminergic neurons in neonatal rats in vivo. Eur J Pharmacol 1991;204:265–72.
[95] Lin M, Walters DE. Dopamine D2 autoreceptors in rats are behaviorally functional at 21 but not 10 days of age. Psychopharmacology 1994;11:262–8.
[96] Shalaby IA, Dendel PS, Spear LP. Differential functional ontogeny of presynaptic receptor regulation. Dev Brain Res 1981;1:434–9.
[97] Becker JB, Robinson TE, Lorens KA. Sex differences and estrus cycle variations in amphetamine-elicited rotational behavior. Eur J Pharmacol 1982;80:65–72.
[98] Handa RJ, Burgess LH, Kerr JE, O'Keefe JA. Gonadal steroid hormone receptors and sex differences in the hypothalamo-pituitary–adrenal axis. Horm Behav 1994;28:464–76.
[99] Handa RJ, Nunley KM, Lorens SA, Louie JP, Mcgivern RF, Bolnow MR. Androgen regulation of adrenocorticotropin and corticosterone secretion in the male rat following novelty and foot shock stressors. Physiol Behav 1994;55:117–24.
[100] Robinson TE, Becker JB, Presty SK. Long-term facilitation of amphetamine-induced rotational behavior and striatal dopamine release produced by a single exposure to amphetamine: sex differences. Brain Res 1982;253:231–41.
[101] Forgie ML, Stewart J. Sex differences in the locomotor-activating effects of amphetamine: role of circulating testosterone in adulthood. Physiol Behav 1994;56:639–44.
[102] Fuller RW, Snoddy HD, Mason NR, Clemens JA, Bemis KG. Elevation of serum corticosterone in rats by dopamine agonists related in structure to pergolide. Neuroendocrinology 1983;36:285–90.
[103] Fuller RW, Snoddy HD. Elevation of serum corticosterone concentrations in rats by pergolide and other dopamine agonists. Endocrinology 1981;109:1026–32.
[104] Casolini P, Kabbaj M, Leprat F, Piazza P, Rouge-Pont F, Angelucci L, Simon H, Le Moal M, Maccari S. Basal and stress-induced corticosterone secretion is decreased by lesion of mesencephalic dopaminergic neurons. Brain Res 1993;622:311–4.
[105] Piazza PV, Le Moal M. Pathophysiological basis of vulnerability to drug abuse: role of an interaction between stress, glucocorticoids, and dopaminergic neurons. Ann Rev Pharmacol Toxicol 1996;36:359–78.
[106] Piazza PV, Le Moal M. The role of stress in drug self-administration. Tr Pharmacol Sci 1998;19:67–74.
[107] Piazza PV, Maccari S, Deminiere JM, Le Moal M, Mormede P, Simon H. Corticosterone levels determine individual vulnerability to amphetamine self-administration. Proc Natl Acad Sci USA 1991;88:2088–92.
[108] Badiani A, Morano MI, Akil H, Robinson TE. Circulating adrenal hormones are not necessary for the development of sensitization to the psychomotor activating effects of amphetamine. Brain Res 1995;673:13–24.
[109] Piazza PV, Deroche V, Deminiere JM, Maccari S, Le Moal M, Simon H. Corticosterone in the range of stress-induced levels possess reinforcing properties: implications for sensation-seeking behaviors. Proc Natl Acad Sci USA 1993;90:11 738–42.
[110] Swerdlow NR, Koob GF, Cador M, Lorang M, Hauger RL. Pituitary–adrenal axis responses to acute amphetamine in the rat. Pharmacol Biochem Behav 1993;45:629–37.
[111] Welker WI. Free versus forced exploration of a novel situation by rats. Psychol Rep 1957;3:95–108.
[112] Griebel G, Belzung C, Misslin R, Vogel E. The free-exploratory paradigm: an effective method for measuring neophobic behaviour in mice and testing potential neophobia reducing drugs. Behav Pharmacol 1993;4:637–44.
[113] Misslin R, Herzog F, Koch B, Ropartz P. Effects of isolation, handling and novelty on the pituitary–adrenal response in the mouse. Psychoneuroendocrinology 1982;7:217–21.
[114] Blanchard RJ, Kelley MJ, Blanchard DC. Defensive reactions and exploratory behavior in rats. J Comp Physiol Psychol 1974;87:1129–33.
[115] Exner M, Clark D. Behaviour in the novel environment predicts responsiveness to *d*-amphetamine in the rat: a multivariate approach. Behav Pharmacol 1993;4:47–56.
[116] File SE, Peet LA. The sensitivity of the rat corticosterone response to environmental manipulations and to chronic chlordiazepoxide treatment. Physiol Behav 1980;25:753–8.
[117] Hennessy MB, Heybach JP, Vernikos J, Levine S. Plasma corticosterone concentrations sensitively reflect levels of stimulus intensity in the rat. Physiol Behav 1979;22:821–5.
[118] Cirulli F, Adriani W, Laviola G. Sexual segregation in infant mice: behavioral and neuroendocrine responses to *d*-amphetamine administration. Psychopharmacology 1997;134:140–52.
[119] Campbell A, Baldessarini RJ, Teicher MH. Decreasing sensitivity to neuroleptic agents in developing rats: evidence for a pharmacodynamic factor. Psychopharmacology 1988;94:46–51.
[120] Andersen SL, Gazzara RS. The ontogeny of apomorphine-induced alterations of neostriatal dopamine release: effects of spontaneous release. J Neurochem 1993;61:2247–55.
[121] Nomura Y, Yotsumoto I, Oki K. Age-related changes in the central catecholaminergic function and its interaction with methamphetamine during postnatal life in the rat. J Pharm Pharmacol 1981;33:264–6.

[122] Shalaby IA, Spear LP. Psychopharmacological effects of low and high doses of apomorphine during ontogeny. Eur J Pharmacol 1980;67:451–9.
[123] Gazzara RA, Andersen SL. The ontogeny of apomorphine-induced alterations of neostriatal dopamine release: effects on potassium-evoked release. Neurochem Res 1994;19:339–45.
[124] Armfred T, Randrup A. Cholinergic mechanism in brain inhibiting amphetamine-induced stereotyped behavior. Acta Pharmacol Toxicol 1968;26:384–94.
[125] Decsi L, Nagy J. Independent GABAergic and cholinergic modulation of apomorphine-induced stereotyped rearing in the rat. Neuropharmacology 1988;27:281–5.
[126] Wickens J. Striatal dopamine in motor activation and reward-mediated learning: steps towards a unifying model. J Neural Transm 1990;80:9–31.
[127] Lehman J, Langer SZ. The striatal cholinergic interneuron: synaptic target of dopaminergic terminals? Neuroscience 1983;10:1105–20.
[128] Henning SJ. Plasma concentrations of total and free corticosterone during development in the rat. Am J Physiol 1978;235:E451–6.
[129] Flickinger CJ, Herr JC, Baran ML, Howards SS. Testicular development and the formation of spermatic granulomas of the epididymis after obstruction of the vas deferens in immature rats. J Urol 1995;154:1539–44.
[130] Rosenfeld P, Suchecki D, Levine S. Multifactorial regulation of the hypothalamic–pituitary–adrenal axis during development. Neurosci Biobehav Rev 1992;16:553–68.
[131] Stone EA, Quatermain D. Greater behavioral effect of stress in immature as compared to mature male mice. Physiol Behav 1988;63:143–5.
[132] Knyck ET, Eisenberg RM. Effect of amphetamine on plasma corticosterone in the conscious rat. Neuroendocrinology 1979;29:110–8.
[133] Kitchen I, Kelly M, Turner M. Dopamine receptor modulation of corticosterone secretion in neonatal and adult rats. J Pharm Pharmacol 1988;40:580–1.
[134] Choi S, Kellogg CK. Adolescent development influences functional responsiveness of noradrenergic projections to the hypothalamus in male rats. Dev Brain Res 1996;94:144–51.
[135] Choi S, Weisberg SN, Kellogg CK. Control of endogenous norepinephrine release in the hypothalamus of male rats changes over adolescent development. Dev Brain Res 1997;98:134–41.
[136] Wurbel H, Staffaucher M, Von Holst D. Stereotypies in laboratory mice: quantitative and qualitative description of the ontogeny of wire gnawing and jumping in Zur:ICR and Zur:ICR nu. Ethology 1996;102:371–85.
[137] Teicher M, Andersen SL, Hostetter JC. Evidence for dopamine receptor pruning between adolescence and adulthood in striatum but not nucleus accumbens. Dev Brain Res 1995;89:167–72.
[138] Teicher MH, Andersen SL, Glod CA, et al. Neuropsychiatric disorders of childhood and adolescence. In: Yudofsky SC, Hales RE, et al., editors. Textbook of neuropsychiatry, 3, 1997. p. 903–41.
[139] Seeman P, Bzowej N, Guan H, Bergeron C, Becker LE, Reynolds GP, Bird ED, Riedere P, Jellinger K, Watanabe S, Tourtellotte WW. Human brain receptors in children and aging adults. Synapse 1987;1:399–404.
[140] Spangler R, Zhou Y, Schlussman SD, Ho A, Kreek MJ. Behavioral stereotypies induced by a binge cocaine administration are independent of drug-induced increases in corticosterone levels. Behav Brain Res 1997;86:201–4.
[141] Herman JP, Cullinan WE. Neurocircuitry of stress: central control of the hypothalamo-pituitary–adrenocortical axis. TINS 1997;20:78–84.
[142] Infurna RN, Spear LP. Developmental changes in amphetamine-induced taste aversions. Pharmacol Biochem Behav 1979;11:31–5.
[143] Ramsay DS, Woods SC. Biological consequences of drug administration: implications for acute and chronic tolerance. Psychol Rev 1997;104:170–93.