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Chronic cocaine or ethanol exposure during adolescence alters novelty-related behaviors in adulthood

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Abstract

Adolescence is a time of high-risk behavior and increased exploration. This developmental period is marked by a greater probability to initiate drug use and is associated with an increased risk to develop addiction and adulthood dependency and drug use at this time is associated with an increased risk. Human adolescents are predisposed toward an increased likelihood of risk-taking behaviors [Zuckerman M. Sensation seeking and the endogenous deficit theory of drug abuse. NIDA Res Monogr 1986;74:59–70.], including drug use or initiation. In the present study, adolescent animals were exposed to twenty days of either saline (0.9% sodium chloride), cocaine (20 mg/kg) or ethanol (1 g/kg) i.p. followed by a fifteen-day washout period. All animals were tested as adults on several behavioral measures including locomotor activity induced by a novel environment, time spent in the center of an open field, novelty preference and novel object exploration. Animals exposed to cocaine during adolescence and tested as adults exhibited a greater locomotor response in a novel environment, spent less time in the center of the novel open field and spent less time with a novel object, results that are indicative of a stress or anxiogenic response to novelty or a novel situation. Adolescent animals chronically administered ethanol and tested as adults, unlike cocaine-exposed were not different from controls in a novel environment, indicated by locomotor activity or time spent with a novel object. However, ethanol-exposed animals approached the novel object more, suggesting that exposure to ethanol during development may result in less-inhibited behaviors during adulthood. The differences in adult behavioral responses after drug exposure during adolescence are likely due to differences in the mechanisms of action of the drugs and subsequent reward and/or stress responsivity. Future studies are needed to determine the neural substrates of these long lasting drug-induced changes.
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1. Introduction

Adolescence is a developmental time period that is characterized by the occurrence of high-risk behavior and increased exploration. This ontogenic period is unique as the brain is undergoing many changes that can have a lasting impact on behavior and cognitive processing (for review see Spear, 2000). Drug use initiation rates are higher during the adolescent period than in any other developmental period. In general, adults who initiate drug use during adolescence are more likely to have higher lifetime rates of drug use and progress to dependency more rapidly than those who began drug use in adulthood (Clark et al., 1998; Helzer et al., 1991; Kandel et al., 1992).

Novelty reactivity/preference is a behavioral trait studied in human and animal models used as a predictor of drug use and potential dependence. A strong relationship between the rewarding aspects of psychomotor stimulants, self-administration rates and novelty preference has been established in animals (Hooks et al., 1992; Klebaur et al., 2001). Rats classified as high responders (HR) to novelty [i.e. exhibit greater locomotor activity in a novel environment] exhibit higher rates of amphetamine and cocaine-induced locomotor activity and self-administer these drugs more readily than low responders (LR) to novelty rats [i.e. exhibit decreased locomotor activity in a novel environment] (Hooks et al., 1991). HR rats engage in greater risk-taking behaviors and

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demonstrate higher behavioral and neurochemical alterations in response to environmental stressors or pharmacological challenges (Bevins et al., 1997; Klebaur et al., 2001). Moreover, dopaminergic responsivity differs between adolescent and adult HR or LR rats (Stansfield and Kirstein, 2005). Overall, these data indicate an association between novelty-seeking and risk-taking behaviors, indicating that high novelty-seeking individuals will be more likely to engage in risky behaviors that can have considerable long-term consequences, such as initiating drug use.

The central nervous system is still developing during adolescence and insults (e.g. chronic drug use) to the brain during this period may play an important role in the increased likelihood to maintain drug use during adulthood (for review, see Spear, 2000. In adult animals, repeated drug exposure produces changes and adaptations at a cellular level that alters the functioning of the entire neural pathway (Kleven et al., 1988). These changes result in the development of complex adaptation such as tolerance, dependence and sensitization (Koob and Le Moal, 1997; Wise, 1980). Chronic cocaine exposure results in functional adaptations such as increased cAMP pathway activity, increased cAMP regulatory element binding protein (CREB) and increased changes in immediate early genes (e.g. FosB) (Nestler and Aghajanian, 1997). In addition, chronic ethanol exposure has been implicated with changes in various postreceptor events of the cAMP signal transduction cascade (i.e., Gs protein, protein kinase A, and CREB) for review, see Uddin and Singh, 2006. Rats injected once a day with cocaine show increased inhibition of dopamine (DA) uptake (Izenwasser and Cox, 1992), whereas rats receiving a continuous infusion of cocaine exhibit attenuated inhibition of DA uptake by cocaine, suggesting changes in duration of drug exposure subsequently induce differential neural changes (Izenwasser and Cox, 1992). Moreover, repeated administration of cocaine produced significant changes in DA during withdrawal. *In vivo* microdialysis studies in the Nucleus Accumbens Septi (NAcc) have shown that once self-administration of cocaine has ended, basal DA levels decrease significantly during this withdrawal period (Parsons et al., 1991). Taken together, these studies in adult animals show that repeated cocaine and ethanol administration result in complex changes in the DA mesolimbic pathway and molecular and cellular changes in the brain that continue long after drug use has stopped. These changes could subsequently impact behavioral phenotypes and lead to a greater vulnerability to drug dependency.

Enduring changes in sustained attention and anhedonia after chronic adolescent ethanol exposure have recently been reported (Slawecki, 2006). Additionally, adolescent ethanol consumption impairs tone conditioning in both male and female rats whereas adult administration had no long-term effects (Smith et al., 2006). These studies are among the first to identify behavioral deficits in adulthood resulting from chronic ethanol exposure in adolescence. To examine long lasting effects of chronic drug exposure during adolescence on novelty-induced behavior in adulthood, the present study assessed responses to a novel context or novel object in a familiar environment. Novelty reactivity was assessed using locomotor activity in the novel environment (i.e. total distance moved on trial 1), total time spent in the center of the open field, novelty preference (i.e. time spent with the novel object) and novel object exploration (i.e. total number of approaches to the novel object). The purpose of the present study was to determine long lasting behavioral differences in adult animals after repeated ethanol or cocaine administration during adolescence.

2. Methods

Forty male Sprague–Dawley (Harlan Laboratories, Indianapolis, IN) rats, offspring of established breeding pairs in the laboratory (University of South Florida, Tampa) were postnatal day (PND) 30 (μ=134 g) at the beginning of the study. No more than one male per litter per age was used in a given condition. Pups were sexed and culled to 10 pups per litter on PND 1. Pups remained housed with their respective dams in a temperature and humidity-controlled vivarium on a 12:12 h light: dark cycle (07:00 h/19:00 h) until PND 21, on PND 21 pups were weaned and male littermates were group housed throughout the entire experiment. Animals were experimentally naive until the beginning of the study (PND 30). The care and use of animals were in accordance with the local standards set by the Institutional Animal Care and Use Committee and the NIH Guide for the Care and Use of Laboratory Animals (NIH, 1989).

2.1. Drug pretreatment

Four experimental groups were included in this study. Beginning on PND 30, animals were injected once per day with either saline [0.9% sodium chloride, i.p., n=9] cocaine hydrochloride [20.0 mg/kg, i.p., n=10] or ethanol [1.0 g/kg, i.p., n=9] in their homecages from PND 30 to 50. To insure injection handling had no effect on saline controls, a naïve control group [n=9] was included that remained uninjected for those 20 days. Following 20 days of drug exposure, animals were withdrawn into adulthood (PND 51–69) when they were tested for novelty preference.

2.2. Procedure

Beginning on PND 66, animals were tested on a black plastic circular platform (116 cm diameter) standing 70 cm from the ground, with a white plastic barrier (48 cm height) enclosing the arena (100 cm diameter). A video camera was suspended directly over the table and recorded the animal's behavior using a Noldus Behavioral Tracking System (Noldus, Netherlands).

Over a period of four consecutive days, each rat (PND 66–69) was placed in the open field in one of the four randomly selected zones and allowed to freely explore the novel environment for 5 min. This procedure was performed twice a day for a total of 8 habituation trials. Immediately following the 8th trial, animals were removed for 1 min while a single novel object (approximately 16 cm high) was attached to the center of the table (trial 9). Rats were placed in a random zone and allowed to explore the familiar environment and novel object for 5 min. Locomotor activity induced by a novel environment (i.e. total distance moved (TDM) on trial 1), time spent in the center of an open field,

novelty preference (i.e. time spent in proximity of the novel object) and novel object exploration (i.e. frequency to approach the novel object) were measured. Novelty preference was defined as time spent within 10.16 cm of the object on trial 9.

3. Results

3.1. Data analyses

Data analyses were performed with Graphpad Prism (Graphpad, CA). The data were expressed as the means+/− SEM, and the significance level was set at $P=0.05$. *T*-tests revealed that naïve and saline pretreated animals did not differ on all measures of activity [$t(13)=0.876$, $p>0.05$ and $t(15)=0.707$, $p>0.05$, respectively] therefore, naïve and saline animals were grouped for all subsequent analyses. Locomotor activity induced by a novel environment (i.e. TDM on trial 1) was analyzed using two-way repeated measures ANOVA with subsequent PLSD post hoc analyses to determine differences across time points and drug conditions. Moreover, three separate one-way ANOVA were performed on time spent in the center of an open field, novelty preference (i.e. time spent with the novel object) and novel object exploration (i.e. frequency to approach a novel object) to assess the effects of adolescent drug exposure. Subsequent post hoc analyses (Dunnett's) were used to isolate differences between drug conditions.

3.2. Cocaine pretreatment

The present findings demonstrate that animals pretreated with cocaine during adolescence exhibited significantly greater locomotor activity induced by a novel environment (i.e. TDM) during the first minute of exposure to the novel environment than did naïve/saline pretreated animals [$F(4,30)=16.71$, $p<0.05$] and spent significantly less time in the center of the open field in the first minute than did naïve/saline animals [$F(2,34)=6.498$, $p<0.05$, $d_D(2,34)=1.85$]. No differences across locomotor activity were detected following the first minute of exposure. Therefore, chronic exposure to cocaine during adolescence increases novelty-induced locomotor activity immediately following exposure to a novel environment and decreases time spent in the center compared to the periphery of the open field in adulthood (see Figs. 1 and 2).

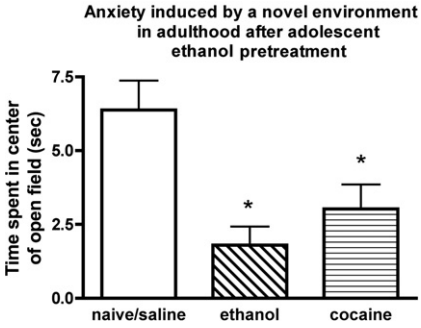

Fig. 2. Adolescent animals pretreated with ethanol (hatched lines) or cocaine (horizontal lines) spent significantly less time in the center of the open field as adults compared to naïve/saline animals. * = differs from naïve/saline.

When tested for novelty preference, animals pretreated with cocaine during adolescence spent significantly less time with the novel object (i.e. decreased novelty preference) compared to naïve/saline or ethanol-pretreated adult animals [$F(2,31)=3.306$, $p<0.05$], [$d_D(2,31)=21.95$] but did not differ in novel object exploration (i.e. frequency to approach the novel object) compared to saline/naïve or ethanol-pretreated rats. Therefore, chronic cocaine during adolescence results in adult animals who spend less time interacting with a novel stimulus compared to naïve/saline or ethanol-pretreated adolescents (see Fig. 3). Because cocaine exerts anorexic effects that might affect activity measures, weights were analyzed across pretreatment conditions. Results indicate that chronic cocaine exposure during adolescence did not significantly alter growth and therefore growth restriction is an unlikely cause of the observed differences in novelty reactivity [$t(38)=0.2936$, $p>0.05$], (see Fig. 4).

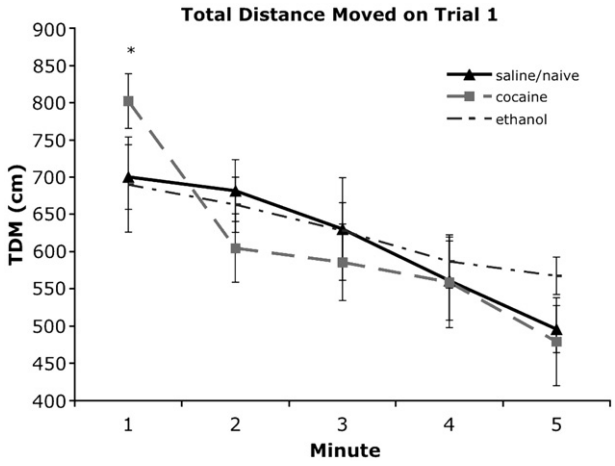

Fig. 1. Adolescent animals pretreated with cocaine (grey square) moved significantly more during the first minute of the first exposure to the novel environment as adults than did naïve/saline (black triangle) or ethanol-pretreated animals (dashed line). * = differs from naïve/saline or ethanol.

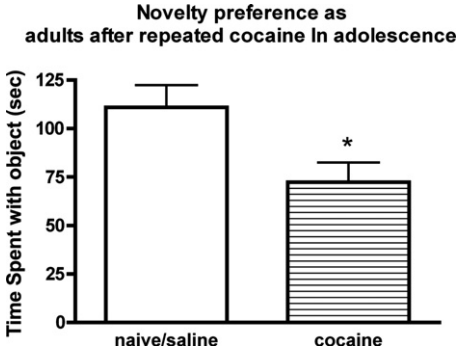

Fig. 3. Adolescent animals pretreated with cocaine (horizontal lines) spent significantly less time with the novel object on trial 9 as adults compared to naïve/saline (white bar) or ethanol-pretreated animals (hatched lines). * = differs from naïve/saline or ethanol.

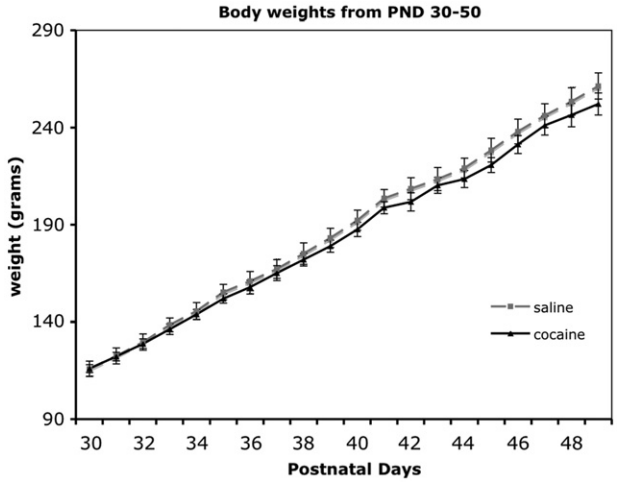

Fig. 4. Adolescent exposure to cocaine compared to saline does not significantly restrict growth due to anorexic effects of high doses of cocaine.

3.3. Ethanol pretreatment

Animals exposed to chronic ethanol during adolescence spent significantly less time in the center of the open field on trial 1 [$F(2,34)=6.498$, $p<0.05$] and exhibited greater novel object exploration than did naïve/saline animals or those exposed to cocaine during adolescence [$F(2,30)=3.775$, $p<0.05$] [$d_D(2,30)=3.825$] and compared to naïve/saline pretreated animals. Additionally, alcohol pretreated animals did not differ in locomotor activity induced by a novel environment, novelty preference or total distance moved on test compared to saline/naïve or cocaine pretreated rats. Chronic ethanol exposure during adolescence increases the tendency of animals to engage in more exploratory or novelty-seeking behaviors (see Figs. 2 and 5).

3.4. High and low novelty preference

To assess the effects of adolescent drug exposure on the phenotypic expression of novelty reactivity in adulthood, a median split was performed on all animals and the distribution of phenotypes assessed for each treatment. Interestingly, adolescent

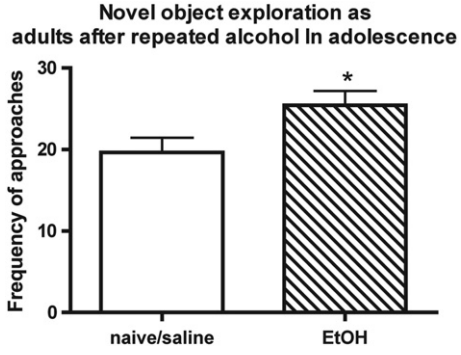

Fig. 5. Pretreatment with ethanol (hatched lines) results in significantly more approaches to a novel object during adulthood than naïve/saline (white bar) or cocaine (horizontal lines) pretreated animals. * = differs from naïve/saline or cocaine.

Table 1
Adolescent exposure to cocaine predisposes a greater percentage of adult animals to be considered LR measured by novelty preference and novel object exploration

	LR	HR
Locomotor activity induced by a novel environment		
Naïve	44%	56%
Saline	50%	50%
Cocaine	33%	66%
Novel object exploration		
Naïve	44%	56%
Saline	56%	44%
Cocaine	45%	55%
Novelty preference		
Naïve	50%	50%
Saline	50%	50%
Cocaine	63%	37%
Anxiety induced by a novel environment		
Naïve	45%	55%
Saline	45%	55%
Cocaine	70%	30%

animals pretreated with cocaine had fewer LR for both novelty preference and novel object exploration (37% and 30%, respectively) than HR (63% and 70%, respectively). In contrast, adolescent cocaine pretreated animals had fewer LR for locomotor activity induced by a novel environment compared to HR (33% and 66%, respectively). This demonstrates that in both the novelty preference and novel object exploration behavioral measures, repeated cocaine during adolescence produces a predisposition towards LR in adulthood, whereas animals exhibit a tendency towards being a HR when measured on novel environment locomotor activity (see Table 1).

4. Discussion

Previous work in humans has demonstrated that individuals who abuse drugs during adolescence are more likely to be dependent on drugs in adulthood (Clark et al., 1998). In addition, novelty preference has been demonstrated to be indicative of a facilitated acquisition of drug use (Klebaur et al., 2001). The aim of the present study was to examine chronic drug exposure (e.g. cocaine or ethanol) during adolescence on the subsequent novelty-induced activity (e.g. TDM on trial 1 and time spent in the center of the open field) and novelty preference (e.g. time spent and approaches) in adulthood.

The present data provide evidence for long-term behavioral changes that endure after chronic drug administration during adolescence. Repeated exposure to cocaine during adolescence modifies the novelty-induced behavioral phenotype in adulthood. Both the novelty preference and novel object exploration behavioral measures following repeated cocaine during adolescence produce a predisposition towards LR in adulthood, whereas animals exhibit a tendency towards being a HR when measured on novel environment locomotor activity; suggesting that animals are more at risk to engage in drug use in adulthood

after adolescent drug exposure due to an alteration in the behavioral phenotype that increases the vulnerability to engage in drug use. Importantly, adult animals exposed to cocaine during adolescence, exhibited greater locomotor activity induced by a novel environment during the first minute of exposure, decreased time spent in the center of a novel environment and decreased novelty preference, which may be indicative of increased stress or anxiety or enhanced neophobia in adulthood after adolescent cocaine. Van den Buuse et al. (2001) have demonstrated that exposure to the novelty of an open field causes an increase in blood pressure, heart rate, body temperature and exploratory locomotor activity, results indicate that an increase in locomotor activity in a novel environment is stressful or anxiogenic. Cocaine has been shown to produce anxiety in human and animal models, either during cocaine administration or during withdrawal. Increased aversion for the illuminated area of the mouse black and white test box model after cocaine exposure was demonstrated by Costall et al. (1989), in addition to increased defensive withdrawal in rats after cocaine exposure (Yang et al., 1992) and a decrease in the number of entries into and time spent in the open arms of an elevated plus maze in mice (Yang et al., 1992). Moreover, following withdrawal from repeated cocaine, animals demonstrated an increase in anxiogenic responses in the elevated plus maze (Sarnyai et al., 1995) and enhanced startle-induced ultrasonic distress vocalizations (Barros, 1996). These data are somewhat counterintuitive as cocaine use and abstinence can induce anxiety in humans and anxiogenic responses in animals and therefore may decrease the appetitive value or motivation for the drug. Some researchers have speculated that the controlled activation of the hypothalamic–pituitary–adrenal (HPA) axis may serve as an arousing stimulus to the animal, very much like novelty-seeking behaviors (Goeders, 2002). Importantly, these studies only investigated the short-term effects of withdrawal after drug exposure whereas the current study examined a longer withdrawal period. Future studies should investigate additional long-term behavioral changes including anxiety related behavioral measures after cessation of chronic cocaine exposure.

The present study also established that adults who were chronically treated with ethanol during adolescence spent less time in the center of the open field during the first minute of trial 1 and had significantly greater novel object exploration, however, these animals did not exhibit a general increase in locomotor activity while the novel object was present. These results suggest that exposure to ethanol during development may result in less-inhibited behaviors during adulthood, and not just a general nonspecific increase in locomotor activity. In humans, several researchers have effectively established a relationship between novelty and/or impulsive behaviors and alcoholism (Dom et al., 2006). However, it can be difficult to establish whether high responders to novelty precede alcohol use or are the result of chronic alcohol use.

These data are interesting as it seems that depending on the mechanism of action of the drug, a different set of behavioral responses is revealed. This is likely due, in part, to differences in the neurotransmitter systems affected. For example, cocaine is a strong catecholamine reuptake inhibitor and has been shown to alter responses in the HPA axis (Kuhn and Francis, 1997). Alternatively, ethanol not only affects DA, but also impacts GABA and long-term ethanol use in adults causes an overall inhibition of the CNS. Long-term exposure to drugs of abuse during adolescence may permanently alter neurocircuitry, making animals more vulnerable to drug use or relapse in adulthood possibly due to behavioral characteristics that facilitate this action.

The present data demonstrate that adolescent animals exposed to drugs of abuse exhibit differential behavioral reactivity in response to novelty as adults, however, the current study only examined a moderate washout period (i.e. 16 days); it is speculated that these behavioral effects are lasting and will endure throughout adulthood, however, future studies are needed to determine if this is the case. Importantly, not only have differences been observed between male and female rats in a novel object conditioned place preference paradigm (Douglas et al., 2003); LR and HR male and female rats differ in the acquisition of sucrose-reinforced responding (Klebaur et al., 2001), stressing the importance that future studies should address differences between male and female animals (possibly due to estrous) and their responsivity to novelty and drugs of abuse.

Novelty preference and risk-taking behaviors have been associated with both an increased propensity to self-administer drugs of abuse and increase drug intake (Bevins et al., 1997; Hooks et al., 1991). The current study demonstrates that chronic adolescent exposure to alcohol may increase responding to novelty as measured by novel object exploration, which subsequently may render the animal more likely to engage in continued drug use [i.e. relapse (see Fig. 5)]. However, these ethanol-pretreated adolescents also spent less time in the center of an open field on trial 1 compared to naïve or saline pretreated animals, suggesting that these animals may be more anxious in the novel environment. Young animals exposed to stress (i.e. maternal separation) exhibited greater ethanol intake as adults as well as exhibiting greater stress responses (Huot et al., 2001; Ploj et al., 2003), suggesting that the reinforcing efficacy of ethanol increases in animals more reactive to stress. Interestingly, chronic adolescent exposure to cocaine produced increased locomotor activity in a novel environment, which based on previous studies suggests that this behavioral characteristic would predispose the animal to drug self-administration (Bevins et al., 1997; Hooks et al., 1991). Conversely, cocaine pretreated animals demonstrated decreased time spent in the center of the open field on trial 1 and decreased novelty preference, it is possible this is an anxiogenic response in these animals compared to naïve or saline pretreated animals and may facilitate drug use. An increase in cocaine self-administration has been observed in stressed or anxious animals (Covington and Miczek, 2005; Marquardt et al., 2004), and chronic cocaine causes an increase in anxiety (Hayase et al., 2005; Rogerio and Takahashi, 1992; Wood and Lal, 1987) providing an explanation for why adolescents exposed to cocaine (who subsequently may be more stressed or anxious) may be more likely to engage in continued drug use. Future studies need to isolate the rewarding efficacy of drugs of abuse in LR and HR animals to novelty.

It is important to mention the difficulty in interpreting the current data as predictive of adolescent specific addiction in the absence of data collected from animals that were exposed to drug in adulthood. Future studies need to address this possibility. Regardless, the differences in behavioral reactivity in adulthood could have implications in the susceptibility to relapse. Some addiction theories state that during drug administration, strong connections between drug cues and the drug experience are strengthened, (possibly modulated by DA) consequently, increasing the likelihood that an individual will relapse when exposed to these drug cues at a later point (Robinson and Berridge, 1993). Moreover, this could be amplified if drug use occurs during adolescence as the brain is still developing. The transition from adolescence to adulthood is a critical developmental period involving the maturation of the mesocorticolimbic circuitry, where not only the development of this system, but the alteration of this system due to pharmacological insult may produce alterations in response to stress and subsequent increased novelty-seeking, and risk-taking behaviors which could result in drug use initiation or relapse (Chambers et al., 2003; Douglas et al., 2003; Spear, 2000).

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