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Locomotor activity changes in female adolescent and adult rats during repeated treatment with a cannabinoid or club drug

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Abstract:
Adolescents and young adults of both sexes are the primary consumers of "club" drugs; yet, most of the mechanistic preclinical research in this area has been performed in adult male rodents. The purpose of this study was to evaluate the acute and repeated effects of drugs that are commonly abused by adolescents in female adolescent and adult rats in a rodent model of behavioral sensitization. During two five-day periods separated by a two-day break, rats were injected daily with saline or with one of the following drugs: cocaine (7 or 15 mg/kg), ketamine (3 or 10 mg/kg), 3,4-methylenedioxymethamphetamine (MDMA) (3, 10, or 30 mg/kg), or Δ^9-tetrahydrocannabinol (THC) (0.03, 0.1, 0.3 or 1 mg/kg) and their locomotor activity was measured. Cocaine increased activity across days in both age groups. Whereas ketamine produced progressive increases in activity with repeated administration in rats of both ages, MDMA increased, and then decreased, activity in the chronic dosing regimen in female adolescents only. Tolerance to the initial stimulatory effects of low doses of THC was observed at both ages. The results with THC are similar to those obtained for male rats tested under identical conditions in a previous study; however, in contrast with the present results in females, male adolescent rats in the previous study failed to develop behavioral sensitization to ketamine. Together, these results suggest that age and sex strongly influence the progressive adaptive changes that occur with repeated administration of some, but not all, of these commonly abused substances.

Key words:
behavioral sensitization, club drugs, cocaine, female rats, locomotor activity, MDMA, THC

Abbreviations: MDMA – 3,4-methylenedioxymethamphetamine, PN – postnatal, THC – Δ^9-tetrahydrocannabinol

Introduction

Historically, the majority of clinical and preclinical research on substance abuse has focused on the physiological and behavioral underpinnings of this health problem on males. Only relatively recently has attention shifted to include females and examination of sex/gender differences has become increasingly common [36]. With the possible exception of investigation of the effects of substance use on pregnant women and their offspring, however, empirical studies of the specific effects of abused substances in women are still in the minority. Yet, epidemiological studies have shown that the patterns of drug use in women differ from those seen in men [16, 18]. For ex-

ample, while women may initially take lower doses of an abused drug, they tend to become addicted faster and to relapse more frequently following a period of abstinence [2], although gender/sex differences have also been observed during other phases of the substance abuse process [7].

Initial experimentation with illicit drugs for both sexes typically begins during adolescence, a time of neuronal reorganization and receptor pruning in the central nervous system [34]. Consequently, adolescent brains differ from those of adults. Further, these differences are superimposed upon rapid sexual differentiation of the brain and behavior that is induced, in part, by the surge in gonadal hormones that occurs during adolescence [30]. In adult female rodents, hormonal status has been shown to strongly influence responses to drugs of abuse [2, 7]. For example, sensitivity to cocaine's locomotor stimulant effects peaks during proestrus and estrus and plunges during diestrus [32]. The behavioral effects of drugs of abuse in adolescents, and particularly in female adolescents, have not been as extensively investigated.

The purpose of the present study was to examine the effects of selected drugs that are commonly abused by adolescents and young adults in a rodent model of behavioral sensitization. In this context, behavioral sensitization is the phenomenon whereby initial drug-induced stimulation of locomotor activity is enhanced following repeated administration of the drug. It is a robust phenomenon that has been observed with drugs of abuse from several distinct classes, including nicotine [3], but especially with psychostimulants and represents a form of neural adaptation [27, 28]. Because behavioral sensitization is believed to be one of the early processes that may occur in the development of drug dependence [28], examination of this effect in adolescents is particularly important. In a previous report, the effects of cocaine, 3,4-methylenedioxymethamphetamine (MDMA), ketamine and Δ^9-tetrahydrocannabinol (THC: primary psychoactive substitutent of marijuana) in male adolescent and adult rats were described [38]. Cocaine was chosen because it is a prototypic psychomotor stimulant that has often been used in behavioral sensitization studies. In this study, it was intended to serve as a positive control. The other three drugs were chosen because they are illicit drugs that are used recreationally particularly during adolescence/early adulthood. In the present study, these drugs were tested under identical experimental conditions and during the same time period in female adolescent and adult rats as in our previous study with male rats.

Materials and Methods

Subjects

Adolescent female Long-Evans rats used as subjects were bred in house using purchased dams and sires (Harlan, Dublin, VA). After breeding, the individually housed dams were left undisturbed except for providing food, water, and fresh bedding until they gave birth (postnatal day 0, PN0). Sufficient woodchip bedding was available in each cage for nesting. Female pups were randomly selected (one per litter) for each of the drug treatment groups described below. On PN21, pups were weaned and were pair-housed with a same-sex rat from another litter that had been assigned to the same drug treatment group. Male pups from the same litters were used in an identical series of experiments that have already been published [38]. Male and female pups that were not used in either study were assigned to other studies. The rat pups were tested for 10 days between ages PN27–PN38. To provide adult subjects for comparison, drug naive adult female rats were ordered (Harlan) at an age of greater than PN65 and were also pair-housed with another female assigned to receive the same drug treatment. Purchased rats were allowed to acclimate to the animal facility for at least one week before initiating testing. Throughout the experiment, all rats, adolescent and adult, were housed in clear plastic shoebox style cages in a temperature-controlled (20–22°C) environment with a 12-hour light-dark cycle (lights on at 7:00 a.m.). All rats had free access to food and water while in their home cage. The studies reported in this manuscript were carried out in accordance with guidelines published in the *Guide for the Care and Use of Laboratory Animals* [24] and were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

Drugs

Cocaine HCl [National Institute on Drug Abuse (NIDA), Bethesda, MD, USA] and 3,4-methylenedioxymethamphetamine (MDMA) [NIDA] were dissolved in saline. Ketamine (Phoenix Scientific, Inc., St. Joseph, MO, USA) was diluted with saline from a commercial stock of 100 mg/ml. THC [NIDA] was mixed in a vehicle of absolute ethanol, Emulphor-620 (Rhone-Poulenc, Inc., Princeton, NJ,

USA), and saline in a ratio of 1:1:18. All injections were administered intraperitoneally at a volume of 1 ml/kg. Except for THC, placement in the locomotor chambers occurred immediately after injection. Rats were placed in the locomotor chambers 30 min after injection with THC.

Apparatus

Clear plastic rat cages (22.5 cm width × 44 cm length × 20 cm height) placed in sound-attenuating cabinets were used as activity chambers. Each cabinet contained up to 12 chambers, with a maximum of 2 chambers per shelf. Activity chambers were free of bedding and were wiped down with a dilute alcohol solution between sessions. Each chamber was placed in a holding rack with 4 × 8 equally spaced photocell beams (4.5 cm from bottom of cage) on the x- and y-axes (Lafayette Instrument, Lafayette, IN) and locomotor activity was measured as total number of beam breaks for the entire session. All activity measurements were performed in darkness (i.e., with the cabinet doors closed).

Procedure

Female pups and adult rats were randomly assigned to receive saline or one dose of one of the test drugs (see drug section). Rat pups in the different dose groups for each drug were chosen from different litters (i.e., no littermates were in the same drug/dose treatment group). Dosing and testing began on PN27 (adolescent rats) or following acclimation (adult rats). Test drugs and doses were as follows: saline, cocaine (7 and 15 mg/kg), ketamine (3 and 10 mg/kg), MDMA (3, 10, and 30 mg/kg), and THC (0.03, 0.1, 0.3, and 1 mg/kg). On test days, each rat was transported to the laboratory, injected with its assigned dose of drug or saline and placed in one of the locomotor chambers for a 20-min session. After the session, the rat was returned to its home cage. For the next 4 days, this sequence of drug injection followed by locomotor activity assessment was repeated. On days 6 and 7 of the experiment (PN32–PN33 for adolescent rats), rats were left undisturbed in their home cages in the vivarium. Subsequently, the rats received 5 more daily injection and testing sessions (PN34–PN38 for adolescent rats). Throughout the dosing regimen, an individual rat was always tested in the same locomotor chamber. The purpose of the two-day break in drug injections was to facilitate the behavioral sensitization (while remaining within the approximately two-week period of adolescence). We have successfully used this procedure in previous studies that have examined behavioral sensitization in adolescent rats [4, 38]. In addition, previous research has shown that intermittent (*vs.* continuous) drug administration has been shown to enhance the development of behavioral sensitization [10, 26].

Data analysis

Spontaneous activity was measured as total number of photocell beam interruptions during the 20-min session. The mean (± SEM) values for the dependent measures were calculated across dose and time for each age separately. Separate mixed factorial ANOVAs (age × dose × repeated time) were performed on data for each drug. When three-way interactions were significant, further analysis was conducted separately in each age group with a mixed factorial ANOVA (dose × repeated time) followed by Tukey-Kramer *post-hoc* tests ($\alpha = 0.05$) to compare individual means.

Results

As shown in Figure 1, cocaine (7 and 15 mg/kg) increased activity compared to saline in both adolescent and adult female rats (Fig. 1, left and right top panels, respectively) initially and across the entire 10 days of dosing [main effect of dose: $F(2,25) = 66.8$, $p < 0.05$]. Activity was not significantly different between the two doses. Although increases in the stimulatory effects of 15 mg/kg cocaine (compared to acute effects of the drug on day 1) were observed, this enhancement was only transient for both age groups, occurring only on study days 4, 5 and 9 [dose × day interaction: $F(18,225) = 2.6$, $p < 0.05$]. Age differences were not apparent for either acute or sub-chronic effects of cocaine.

Ketamine also increased activity in female rats of both ages (Fig. 1, left and right bottom panels); however, the time course of ketamine's stimulatory effects differed between adolescent and adults rats [age × dose × day interaction: $F(18,216) = 5.9$]. Acutely, ketamine did not alter level of activity at either dose or at either age (compared to rats that received saline). With sub-chronic administration, 10 mg/kg ketamine

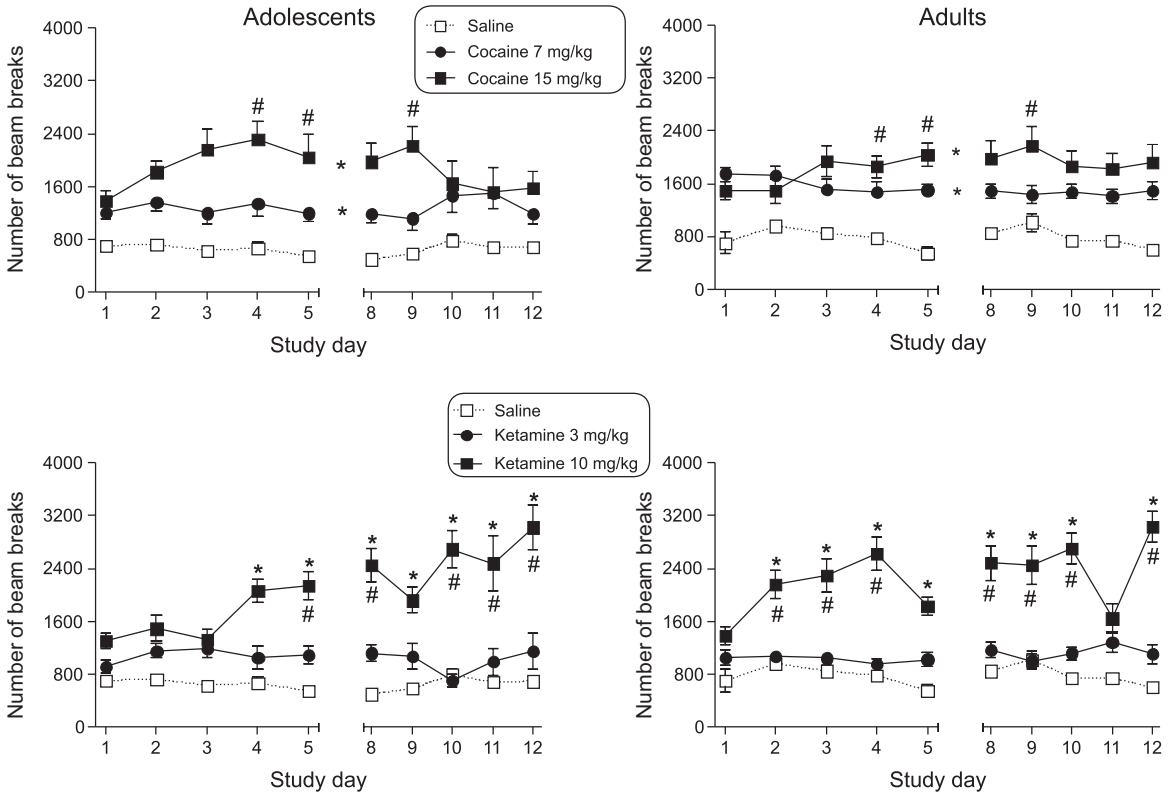

Fig. 1. Effects of cocaine (top panels) and ketamine (bottom panels) on locomotor counts (number of beam breaks) in female rats during adolescence (left panels) and in adulthood (right panels) over a 10-day period. Test sessions occurred daily for 5 consecutive days starting on PN27 in adolescents or during adulthood (> PN65) and then were resumed following a 2-day hiatus. Points represent the mean (± SEM). For all means, n = 9–11, except for n = 6 for 3 mg/kg ketamine. In the top panels (cocaine), * indicates significant main effect of dose ($p < 0.05$) *vs.* saline control group and # indicates significant main effect of day ($p < 0.05$) *vs.* day 1. In the bottom panels (ketamine), * indicates significant time × dose interaction with *post-hoc* difference ($p < 0.05$) *vs.* respective saline control group. # Indicates significant time × dose interaction with *post-hoc* difference ($p < 0.05$) compared to acute effects of the dose (i.e., on study day 1)

produced rapid changes in activity level in adult females (Fig. 1, bottom right panel), with significant activity enhancement (compared to saline) and increased activity (compared to its acute effects) during most of the subsequent test sessions [dose × day interaction in adults only: $F(18,243) = 5.9$, $p < 0.05$]. In contrast, adolescent females exhibited a slower onset of ketamine-induced stimulation (Fig. 1, bottom left panel), with activity increases (compared to saline) not observed until PN30 (study day 4) [dose × day interaction in adolescents only: $F(18,243) = 5.7$, $p < 0.05$]. Once ketamine induced increases in activity, however, enhancement of these effects (compared to PN27, study day 1) was steady and occurred during most subsequent test sessions.

Figure 2 shows the results of MDMA administration in female adolescent and adult rats (Fig. 2, left and right top panels, respectively). The effects of three doses of MDMA (3, 10 and 30 mg/kg) were initially assessed in rats of both ages; however, dosing with 30 mg/kg MDMA could not be completed in the adult females due to development of toxicity (including lethality) within the first week of administration. In contrast, no overt signs of toxicity of this high dose of MDMA were noted in female adolescents and the entire dosing regimen was completed in all adolescent rats, with activity increases (compared to saline) observed for this dose across all days [dose × day interaction in adolescents only: $F(27,215) = 3.1$, $p < 0.05$]. Administration of lower dose(s) of MDMA increased activity in rats of both ages. Whereas 10 mg/kg MDMA increased activity (compared to saline) during most sessions in adolescent and adult rats [dose × day interaction in adults: $F(18,171) = 1.8$, $p < 0.05$ and in adolescents: $F(27,215) = 3.1$, $p < 0.05$], the 3 mg/kg dose of MDMA increased activity only in adolescents and only on a single day (PN35, study day 8). Adaptation to the initial stimulatory effects of MDMA did not occur in adult rats at ei-

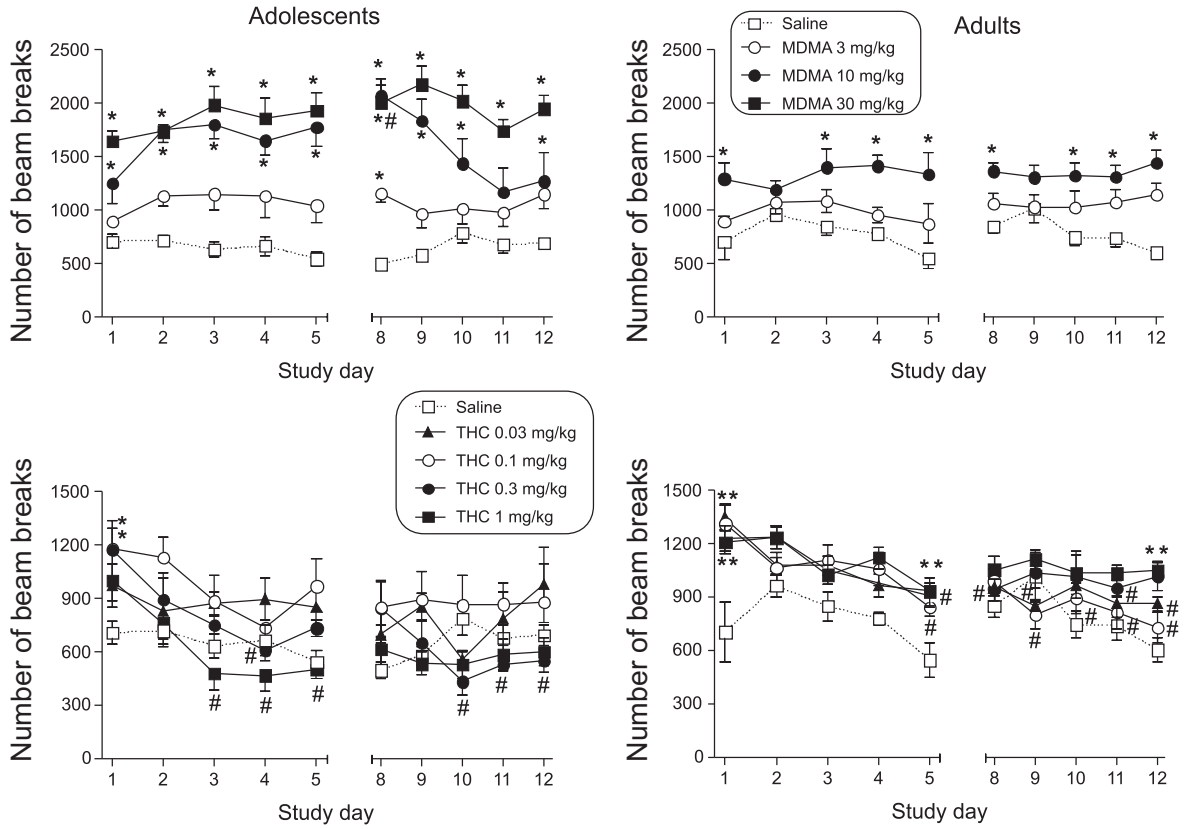

Fig. 2. Effects of MDMA (top panels) and THC (bottom panels) on locomotor counts (number of beam breaks) in female rats during adolescence (left panels) and in adulthood (right panels) over a 10-day period. Test sessions occurred daily for 5 consecutive days starting on PN27 in adolescents or during adulthood (> PN65) and then were resumed following a 2-day hiatus. Points represent the mean (± SEM). For all means, n = 5–6. * Indicates significant time × dose interaction with *post-hoc* difference ($p < 0.05$) *vs.* respective saline control group. # Indicates significant time × dose interaction with *post-hoc* difference ($p < 0.05$) compared to acute effects of the dose (i.e., on study day 1). Note: range of y-axes differ for panels depicting results for MDMA and THC

ther MDMA dose. In adolescent rats, 10 mg/kg MDMA increased activity over the course of the first week to a significant (compared to PN27, study day 1) peak on PN34 (study day 8); however, this activity enhancement was transient, dissipating during the latter part of the second week of dosing.

Figure 2 also shows the effects of THC on locomotor activity in adolescent and adult female rats (Fig. 2, left and right bottom panels, respectively). Doses of THC differentially affected activity dependent upon age and day [age × dose × day interaction: $F(36,519) = 2.2$, $p < 0.05$]. Although the activity level after the initial saline injection was similar across age, the range of acute doses of THC that stimulated activity was wider for adults than adolescents, with all doses increasing activity acutely in adults [dose × day interaction for adults only: $F(36,261) = 2.6$, $p < 0.05$] whereas only the 0.1 and 0.3 mg/kg doses did so in adolescents [dose × day interaction for adolescents only: $F(36,258) = 2.1$, $p < 0.05$]. At both ages, however, sub-chronic administration of THC produced rapid tolerance to its stimulatory effects with activity levels not significantly different from vehicle by the second test day. Further, decreases in activity (compared to acute effect on study day 1) were observed during later days in the dosing regimen in rats of both ages (at doses of 0.3 and 1 mg/kg in adolescents and at doses of 0.03 and 0.1 mg/kg in adults).

Discussion

Numerous studies have demonstrated that robust cocaine-induced behavioral sensitization to cocaine's acute stimulatory effects on locomotor activity devel-

ops in adult male rodents following repeated administration [10, 17, 26]. Later studies showed that behavioral sensitization also consistently occurred in male adolescent rats, albeit relative sensitivity to this effect across age depends upon the specific conditions used to induce behavioral sensitization [8, 13, 20]. Although female adolescent and adult rats in the present study also exhibited enhancement of initial locomotor stimulation with a regimen of repeated cocaine administration identical to one used to induce behavioral sensitization in male rats of both ages [38], it was transient in nature rather than sustained. These results are in contrast with those of previous studies, in which reliable cocaine-induced behavioral sensitization in adult female rats was reported [22, 33, 41]. Several factors may have contributed to the finding of less enduring behavioral sensitization in female rats in the present study. For example, whereas most previous studies that have examined age and sex differences in cocaine behavioral pharmacology used Sprague-Dawley or Wistar rats [9, 13, 21, 29], the present experiments were conducted in Long-Evans rats. Between- and within-strain variability in sensitivity to cocaine's stimulatory effects have been reported, the latter in adult rats of both sexes [15, 22]. Interestingly, with repeated cocaine injection, behavioral sensitization to these effects developed in female Sprague-Dawley rats regardless of the rats' level of sensitivity to the initial effect [22], suggesting that this strain may have an overall high propensity to display cocaine-induced behavioral sensitization. Gonadal hormone levels are also important modulators of behavioral response to cocaine, with estrogen-induced decreases in acute locomotor activity and enhancement of behavioral sensitization following repeated cocaine administration reported [31, 33]. In this regard, it is interesting to note that female adolescent rats that received cocaine (especially the 15 mg/kg dose) exhibited greater variability in locomotor activity across days than did female adult rats, suggesting that hormonal levels may be more labile in this younger group. Since the intact female rats used in this study were not monitored for hormonal status, however, definitive determination of whether or not these factors altered underlying processes related to development of behavioral sensitization to cocaine here was not possible.

In contrast with the inconsistent pattern of activity across repeated administration that was observed for cocaine in female rats, ketamine-induced behavioral sensitization occurred by the end of the first week of dosing in female rats of both ages and was especially pronounced in the younger rats. Classified as a dissociative anesthetic, ketamine (Special K, Vitamin K, K) acts primarily as a noncompetitive N-methyl-D-aspartate (NMDA) receptor channel blocker to produce, at subanesthetic doses, a dissociative state characterized by sensory distortion and sedative properties [19]. Previous studies have shown that some of the effects of ketamine and other NMDA channel blockers are sex-dependent. Whereas female adolescent and adult rats were equally sensitive to the development of behavioral sensitization in the present study, adolescent male rats exhibited decreased sensitivity to ketamine-induced behavioral sensitization compared to their adult counterparts when tested under conditions identical to those used with the female rats [38]. Enhanced responsiveness of female (*vs.* male) rats across ages to the acute effects of noncompetitive NMDA receptor channel blockers on locomotor activity also has been reported previously [12, 40]. To the extent that behavioral sensitization reflects underlying adaptation of neural circuits related to brain reward [e.g., as suggested by 28, 37], these results suggest that female adolescents may be more susceptible than female adults and males of either age to initial changes that may accompany the development of drug dependence. On the other hand, ketamine-induced memory impairment has been shown to be greater in adult male (*vs.* female) humans [23], suggesting that sex and age differences in sensitivity to ketamine's effects may also depend upon the measure.

The "club drug" MDMA (XTC, ecstasy, X, Adam) increases the carrier-mediated release and inhibition of reuptake of 5-HT and DA when administered acutely and decreases synthesis and reuptake of 5-HT with chronic use, effects that have been shown to be neurotoxic to these neurons in multiple species [11]. Further, adult rats show greater sensitivity to these MDMA-induced neurotoxic effects than do adolescent rats [5]. Consistent with the latter report, adult female and male rats [present study and 38, respectively] exhibited increased lethality of MDMA, with deaths occurring in adult rats within a few exposures to the 30 mg/kg dose. In female adolescent rats, however, this dose of MDMA produced sustained increases in locomotor activity without overt signs of toxicity, as it did previously in male adolescent rats [38]. The 10 mg/kg dose of MDMA increased activity acutely and across repeated administration in female

rats of both ages; however, it did not produce behavioral sensitization, although a brief enhancement of activity occurred (1 session) in adolescent females, with activity returning to previous levels rapidly over the next couple of administrations. These effects are generally consistent with the lack of behavioral sensitization reported in male adolescent rats [1, 38], as well as with previous reports that females are sensitive to MDMA-induced locomotor stimulation [25, 35].

Similar to cocaine and MDMA, acute THC increased locomotor activity. Further, increased activity upon initial THC exposure occurred over a wider dose range in female adult rats than in female adolescent rats. At both ages, however, a return to activity levels seen in saline-treated rats was observed upon repeated THC administration. These results are consistent with development of tolerance (*vs.* behavioral sensitization) and resemble those previously obtained with male adolescent and adult rats administered THC under the same dosing regimen [38]. Rapid tolerance development upon repeated administration is characteristic of many other pharmacological effects produced by cannabinoids, including high dose effects such as hypothermia, antinociception, catalepsy, and suppression of activity [for a review, see 14], and has been observed in adolescent rats of both sexes [39]. In the present study, tolerance to the stimulatory effects of the lower doses of THC developed faster and tended to be more pronounced in female adolescent rats than in female adult rats. Interestingly, desensitization of cannabinoid CB_1 receptors (the primary site for mediation of THC's psychoactive effects) was greater in several brain regions in female adolescent rats as compared to female adult rats [6], suggesting a possible pharmacodynamic mechanism for the greater magnitude of tolerance in female adolescent rats observed here.

In summary, the results of this study show that acute and repeated administration of cocaine, ketamine, and THC produce patterns of activity changes that are similar across age in female adolescent and adult rats. In contrast, the effects of MDMA on locomotor activity depended upon age, with adult female rats exhibiting stimulation only whereas adolescent female rats showed a biphasic pattern of increases followed by decreases in activity over repeated administration. MDMA was also less toxic in the latter group. The most prominent difference between results obtained in female rats and those obtained in male rats tested under identical conditions in a previous study was that adolescent male rats did not develop sensitization to ketamine [38]. To the extent that sensitization serves as a model of the transition from use to abuse, these results suggest that females may be more sensitive to progression to abuse with repeated use of ketamine than males. Further, the divergent nature of the findings across age and sex emphasize the importance of inclusion of these variables in investigation of the behavioral mechanisms underlying substance abuse.

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