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Importance of environmental context for one- and three-trial cocaine-induced behavioral sensitization in preweanling rats

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Abstract

Rationale—Prewanling rats, unlike adults, exhibit context-independent behavioral sensitization after a single pretreatment injection of cocaine.

Objective—The purpose of this study was to examine environmental factors modulating one- and three-trial sensitization in preweanling rats.

Methods—For preweanling rats, drug pretreatments occurred on PD 17–PD 19 (Experiment 1) or PD 19 (Experiment 2). One set of rats was injected with cocaine (30 mg/kg) and placed in anesthesia (“small”), operant conditioning (“large”), or activity chambers for 30 min. Rats were returned to the home cage and injected with saline. Additional groups of rats were injected with saline and placed in small, large, or activity chambers for 30 min and then injected with cocaine after being returned to the home cage. Control groups were injected with saline at both time points. In separate experiments, rats were pretreated with cocaine or saline and restricted to the home cage. On PD 20, all rats were injected with cocaine (20 mg/kg) and placed in activity chambers where locomotor activity was assessed for 60 min. For comparison purposes, sensitization was also assessed in adult rats.

Results—Adult male and female rats exhibited only context-dependent sensitization, whereas preweanling rats showed context-independent sensitization in a variety of conditions (e.g., when pretreated with cocaine in various novel chambers or the home cage).

Conclusions—These results suggest that nonassociative mechanisms underlying behavioral sensitization are functionally mature in preweanling rats, but associative processes modulating the strength of the sensitized response do not function in an adult-like manner during the preweanling period.

Keywords

Behavioral sensitization; Cocaine; Ontogeny; Environmental context; Inhibitory conditioning

Introduction

Behavioral sensitization occurs when rats pretreated with a psychostimulant drug (e.g., cocaine or amphetamine) show an augmented behavioral response after a challenge injection of the

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same drug (Robinson and Becker 1986; Kalivas and Stewart 1991). In a typical experiment adult rats are given repeated daily administrations of a psychostimulant and are then tested for behavioral sensitization after seven or more drug abstinence days. In this circumstance sensitized responding is more robust when drug pretreatment and testing occur in the same novel environment (Badiani et al. 1997; Carey and Gui 1998; Tirelli and Terry 1998). Even so, behavioral sensitization can be detected if the psychostimulant was not previously paired with the testing chamber (i.e., context-independent sensitization) (Vezina and Stewart 1990; Browman et al. 1998a, b; Partridge and Schenk 1999).

Interestingly, adult rats and mice are capable of exhibiting behavioral sensitization after a single pretreatment administration of cocaine or amphetamine (i.e., one-trial sensitization). Using this experimental methodology the importance of environmental factors appears to be maximized (White et al. 1998) because only context-dependent behavioral sensitization, but not context-independent sensitization, is evident (Weiss et al. 1989; Fontana et al. 1993; Jackson and Nutt 1993; Battisti et al. 2000; McDougall et al. 2005, 2007). For example, Battisti et al. (1999a, b) gave adult mice a single injection of amphetamine or apomorphine and then restricted them to chambers that differed according to size, color, and texture of the bedding. When challenged with the same drug one day later, only mice pretreated and tested in the identical or nearly identical environment exhibited a sensitized stereotypic response. Using a somewhat different pretreatment methodology (i.e., care was taken to equate exposures to the test environment on the pretreatment day), Weiss et al. (1989) reported that a sensitized locomotor response was only evident when cocaine pretreatment and testing occurred in the identical (or nearly identical) novel environment. Regardless of procedure, adult rats and mice did not exhibit behavioral sensitization when they were pretreated with a psychostimulant in the home cage (Weiss et al. 1989; Battisti et al. 1999a; see also Fontana et al. 1993; Jackson and Nutt 1993; Battisti et al. 2000; McDougall et al. 2007).

Preweanling rats respond very differently than adults when tested using a one-trial sensitization procedure. Specifically, rats pretreated with cocaine on postnatal day (PD) 19 and tested on PD 20 showed context-independent behavioral sensitization (McDougall et al. 2007, 2009). In these studies, behavioral sensitization was assessed in groups given the same number of exposures to the test chamber as well as cocaine administrations, but only one group was pretreated with cocaine in the test environment (i.e., the other group was injected with cocaine in the home cage). Although an economical and oft used procedure (e.g., Weiss et al. 1989; Fontana et al. 1993; Wood et al. 1998; Zavala et al. 2000), it is not ideal for determining environmental conditioning factors necessary for the expression of behavioral sensitization. Therefore, in the present study we manipulated both environmental context and number of drug pretreatment days (1 vs 3) to determine the importance of environmental conditioning for the expression of behavioral sensitization in preweanling rats. For comparison purposes, the sensitized responding of adult male and female rats was assessed after one pretreatment day.

Materials and methods

Subjects

A total of 224 male and female preweanling rats and 84 male and female adult rats were used. Rats were of Sprague-Dawley descent (Charles River, Hollister, CA) and were raised at California State University, San Bernardino (CSUSB). Litters were culled to ten pups at three days of age. All rats were housed on racks in large polycarbonate maternity cages (56 × 34 × 22 cm) with wire lids and Tek-Fresh® bedding (Harlan, Indianapolis, IN). Prewanling rats were kept with the dam and littermates throughout behavioral testing, whereas postweanling rats were group housed according to sex in maternity cages. Food and water was freely available. The colony room was maintained at 22°–24°C and kept under a 12 L:12 D cycle, with behavioral testing occurring during the light phase of the cycle. Subjects were cared for

according to the “Guide for the Care and Use of Mammals in Neuroscience and Behavioral Research” (National Research Council 2003) under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

Apparatus

Preweanling rats were pretreated in small animal anesthesia chambers (model: PY8 50-0108, Harvard Apparatus, Holliston, MA), standard mouse operant conditioning chambers (Coulbourn Instruments, Allentown, PA), and activity monitoring chambers (Coulbourn Instruments); whereas, adult rats were exclusively pretreated in activity monitoring chambers. The anesthesia chambers (hereafter referred to as “small” chambers) were made of clear Plexiglas with a sliding lid ($23.5 \times 10 \times 10$ cm, $L \times W \times H$). The operant conditioning chambers (hereafter referred to as “large” chambers) had metal walls on three sides, a black Plexiglas wall on the fourth side, a shock grid floor, two inactive levers, an empty food aperture, and a house light that was constantly lit. The large chambers ($18 \times 18 \times 30$ cm, $L \times W \times H$) were placed inside sound-attenuating cubicles equipped with an exhaust fan that provided masking noise. The activity chambers had acrylic walls, a gray plastic floor, and an open top. Different sized activity chambers were used at each age (preweanling rats, $25.5 \times 25.5 \times 41$ cm; adult rats, $41 \times 41 \times 41$ cm) to somewhat control for differences in body size. Each activity chamber included an X–Y photobeam array, with 16 photocells and detectors, that was used to measure horizontal locomotor activity (distance traveled). The position of each rat was determined every 100 msec.

Drugs

(–)-Cocaine hydrochloride (Sigma, St. Louis, MO) was dissolved in saline and injected intraperitoneally (IP) at a volume of 5 ml/kg for preweanling rats and 1 ml/kg for adult rats. Cocaine was always administered at 30 mg/kg on the pretreatment day and 20 mg/kg on the test day. This cocaine regimen produces strong one-trial behavioral sensitization in preweanling rats (McDougall et al. 2007, 2009).

Procedure

Three pretreatment days: preweanling rats—On PD 17, male and female rats ($N = 96$) were randomly assigned to one of twelve pretreatment conditions (Table 1). Rats in the Cocaine-Activity group were taken to the test room and injected with cocaine (30 mg/kg, IP) before being placed in activity chambers for 30 min. These rats were then returned to the home cage and injected with saline after an additional 30 min. The Saline-Activity group was injected with saline before being placed in the activity chamber and then injected with cocaine 30 min after being returned to the home cage. The Activity-Control group received saline at both time points. This procedure was repeated on PD 18 and PD 19, with distance traveled being measured in the activity chambers each day. On PD 17–PD 19, rats assigned to the “large” chambers (i.e., operant conditioning chambers) were taken to a different novel room and placed in the large chambers (distance traveled was not measured). Specifically, rats in the Cocaine-Large group were injected with cocaine (30 mg/kg, IP) immediately before being placed in the large chambers for 30 min, and then injected with saline 30 min after being returned to the home cage. Rats in the Saline-Large group were injected with saline before being placed in the large chambers and then injected with cocaine in the home cage. The Large-Control group received saline at both time points. Rats assigned to the “small” chambers (i.e., anesthesia chambers) were treated identically to the groups just described, with the exception that rats in the Cocaine-Small, Saline-Small and Small-Control groups were taken to a different novel room and placed in the small chambers (distance traveled was not measured). Rats were restricted to the small chambers for 30 min after the first injection, while the second injection was given 30 min after rats were returned to the home cage. Rats in the Tri-Chamber groups

(i.e., the Cocaine-Tri, Saline-Tri, and Tri-Control groups) were treated in the same manner except that each rat was assigned to a different chamber (activity, large, and small) on each of the pretreatment days (i.e., PD 17–PD 19). The sequence in which rats were exposed to the chambers was counterbalanced.

To determine the occurrence of behavioral sensitization, all rats were taken to the test room and injected with cocaine (20 mg/kg, IP) on PD 20. After being injected, rats were immediately placed in activity chambers where distance traveled was measured for 60 min. Thus, rats pretreated with cocaine or saline in the large, small, or activity chambers on PD 17–PD 19 were tested for behavioral sensitization in activity chambers on PD 20.

Separate groups of male and female preweanling rats ($N = 16$) were restricted to the home cage throughout the pretreatment phase. On PD 17–PD 19, rats in the Cocaine-Home group were injected with cocaine in the home cage followed, 60 min later, by an injection of saline in the home cage (half of the rats received the saline injection first followed by the cocaine injection). The Saline-Home group received saline at both time points. On PD 20, all rats were injected with cocaine (20 mg/kg, IP) and immediately placed in activity chambers, where distance traveled was measured for 60 min.

One pretreatment day: preweanling rats—On PD 19, male and female rats ($N = 72$) were randomly assigned to one of nine pretreatment conditions: Cocaine-Activity, Saline-Activity, Activity-Control, Cocaine-Large, Saline-Large, Large-Control, Cocaine-Small, Saline-Small, or Small-Control (Table 1). The experiment was identical to the one just described with two exceptions. First, the pretreatment phase occurred on a single day (i.e., PD 19) and, second, the tri-chamber groups were not included. One day later (i.e., on PD 20), all rats were injected with cocaine (20 mg/kg, IP) and distance traveled was measured in activity chambers for 60 min.

Separate litters of male and female preweanling rats ($N = 40$) were maintained in the home cage on the pretreatment day. On PD 19, rats in the Cocaine-Home group were injected with cocaine in the home cage followed, 60 min later, by an injection of saline in the home cage (the injection sequence was counterbalanced). The Saline-Home group received saline at both time points. On PD 20, cocaine (20 mg/kg, IP) was administered to all rats and distance traveled was measured in activity chambers for 60 min.

One pretreatment day: adult rats—On PD 79, male and female rats ($N = 48$) were randomly assigned to the Cocaine-Activity, Saline-Activity, and Activity-Control groups (Table 2). The Cocaine-Activity group received cocaine (30 mg/kg, IP) immediately before being placed in the test chamber, the Saline-Activity group received cocaine 30 min after being returned to the home cage, and the Activity-Control group received saline at both time points. On PD 80, all rats were injected with cocaine (20 mg/kg, IP) and distance traveled was measured for 60 min.

An additional 38 adult male and female rats were randomly assigned to the Cocaine-Home and Saline-Home groups (procedures pertaining to these groups are described above). Drug pretreatment occurred on PD 79 and the test day was on PD 80.

Data analysis

Litter effects were controlled through both experimental design and statistical procedures. In most experiments no more than one subject per litter was assigned to a particular group. In cases where this procedure was not possible (e.g., analysis of the pretreatment phase), a single litter mean was calculated from multiple littermates assigned to the same group (Holson and Pearce 1992; Zorrilla 1997). When possible, litter was used as the unit of analysis for statistical

purposes (Zorrilla 1997). With this statistical model each litter, rather than each rat, is treated as an independent observation (i.e., a within analysis using one value/condition/litter). Between-subjects statistical procedures were used when analyzing data of adult rats or when experiments included more than ten groups (i.e., when individual litters did not contain enough subjects to provide one subject per group). Preliminary analyses indicated that distance traveled data did not differ according to sex at PD 20, so this variable was removed from subsequent analyses involving preweanling rats.

For all experiments, omnibus repeated measures analyses of variance (ANOVAs) were used for the statistical analysis of distance traveled data. Because each experiment included distinct subsets of treatment groups (e.g., the Cocaine-Activity, Saline-Activity, and Activity-Control groups would constitute a subset), smaller two-way ANOVAs were used when appropriate. When the assumption of sphericity was violated, as determined by Mauchly's test of sphericity, the Huynh-Feldt epsilon statistic was used to adjust the degrees of freedom (Huynh and Feldt, 1976). Corrected degrees of freedom were rounded to the nearest whole number and are indicated by a superscripted "a". Post hoc analysis of distance traveled data was done using Tukey tests ($P < 0.05$).

Results

Three pretreatment days: preweanling rats

Pretreatment phase (PD 17–PD 19)—During the pretreatment phase, the pattern of distance traveled scores differed from the first day to the latter two days (Fig. 1). On PD 17, cocaine-treated rats had significantly greater distance traveled scores than saline controls on all six time blocks [^aDrug $\times$ Day $\times$ Time interaction, $F_{9,64}=5.78$, $P < 0.001$]. On PD 18 and PD 19, however, cocaine (30 mg/kg) only enhanced distance traveled scores on time blocks 1 and 2, because cocaine-treated rats exhibited a substantial decline in locomotor activity as each session progressed.

Test day (PD 20)—Among rats pretreated in the activity chambers (upper left graph, Fig. 2), the Cocaine-Activity and Saline-Activity groups had greater distance traveled scores than the Activity-Control group on the test day [Condition main effect, $F_{2,21}=8.18$, $P < 0.01$]. Sensitized responding was also evident in one of the large chamber groups (lower left graph, Fig. 2), with the Saline-Large group having significantly greater distance traveled scores than the Large-Control group [Condition main effect, $F_{2,21}=5.19$, $P < 0.05$]. Rats in the small chamber groups exhibited behavioral sensitization regardless of whether cocaine pretreatment occurred in the small chamber or the home cage, because the test day distance traveled scores of the Cocaine-Small and Saline-Small groups were significantly greater than the Small-Control group (upper right graph, Fig. 2) [Condition main effect, $F_{2,21}=10.15$, $P < 0.001$]. In the latter case, the Saline-Small group differed from the Cocaine-Small group on time blocks 1–3 and 7–12, and from the Small-Control group on time blocks 2–3 and 8–12 [^aCondition $\times$ Time interaction, $F_{11,113}=2.58$, $P < 0.01$]. Rats assigned to different chambers on successive pretreatment days (i.e., the Tri-Chamber groups) also showed behavioral sensitization (lower right graph, Fig. 2), as both the Cocaine-Tri and Saline-Tri groups had greater distance traveled scores than the Tri-Control group [Condition main effect, $F_{2,21}=9.86$, $P < 0.001$].

Cocaine-pretreated rats restricted to the home cage on PD 17–PD 19 exhibited a sensitized response when challenged with cocaine in the activity chambers on PD 20 (Fig. 3). Specifically, rats in the Cocaine-Home group had greater test day distance traveled scores than rats in the Saline-Home group [Condition main effect, $F_{1,7}=43.74$, $P < 0.001$].

One pretreatment day: preweanling rats

Pretreatment phase (PD 19)—On the pretreatment day, preweanling rats injected with cocaine ($\bar{x} = 7,824$ cm, $SEM = \pm 638$) prior to being placed in the activity chambers had significantly greater distance traveled scores than rats injected with saline ($\bar{x} = 3,241$ cm, $SEM = \pm 434$) [Drug main effect, $F_{1,7}=27.43$, $P<0.01$]. This effect did not vary according to time block.

Test day (PD 20)—Overall, test day distance traveled scores varied according to treatment condition [$F_{8,56}=2.84$, $P<0.01$] and time block [$^aF_{6,44}=26.61$, $P<0.001$]. In the latter case, distance traveled scores showed a general decline as the testing session progressed. Among the groups pretreated and tested in the activity chambers (upper graph, Fig. 4), preweanling rats in the Cocaine-Activity and Saline-Activity groups had greater distance traveled scores than rats in the Activity-Control group [Condition main effect, $F_{2,14}=4.68$, $P<0.05$]. Thus, rats in the activity groups exhibited behavioral sensitization regardless of whether cocaine pretreatment occurred in the activity chamber or home cage. Among the groups pretreated in the large chambers (middle graph, Fig. 4), rats in the Cocaine-Large group exhibited significantly more locomotor activity than rats in the Large-Control group, with the Saline-Large group being intermediate between the two [Condition main effect, $F_{2,14}=4.02$, $P<0.05$]. Likewise, rats pretreated with cocaine either before or after placement in the small chambers exhibited a sensitized locomotor response on the test day (lower graph, Fig. 4), because the Cocaine-Small and Saline-Small groups had greater distance traveled scores than the Small-Control group [Condition main effect, $F_{2,14}=7.16$, $P<0.01$].

Preweanling rats injected with cocaine and restricted to the home cage on the pretreatment day exhibited a sensitized response on the test day (Fig. 5). Specifically, test day distance traveled scores of rats in the Cocaine-Home group were significantly greater than the Saline-Home group (these two groups were not transported to a novel room or placed in a novel chamber on the pretreatment day, but were tested in activity chambers on the test day) [Condition main effect, $F_{1,7}=16.16$, $P<0.001$].

One pretreatment day: adult rats

Pretreatment phase (PD 79)—Overall, adult female rats had greater distance traveled scores than male rats on the pretreatment day (Fig. 6) [Sex main effect, $F_{1,44}=7.90$, $P<0.01$]. When collapsed across sex, adult rats injected with cocaine exhibited substantially greater distance traveled scores than rats injected with saline [Drug main effect, $F_{1,44}=95.10$, $P<0.001$]. The distance traveled scores of cocaine-treated rats did not vary significantly across the six time blocks, whereas distance traveled scores of saline-treated rats showed a progressive decline across the testing session [a Drug $\times$ Time interaction, $F_{4,198}=18.80$, $P<0.001$].

Test day (PD 80)—Test day administration of cocaine (20 mg/kg) stimulated greater distance traveled scores in female rats than male rats [Sex main effect, $F_{1,42}=31.94$, $P<0.001$], an effect that did not vary according to treatment condition (Fig. 7) [Sex $\times$ Condition interaction, $F_{2,42}=0.90$, $P=0.41$]. Adult rats exhibited behavioral sensitization after a single drug pretreatment, but only when cocaine was administered in the novel activity chamber. Specifically, rats in the Cocaine-Activity group had greater distance traveled scores than rats in the Saline-Activity and Activity-Control groups on the test day [Condition main effect, $F_{2,42}=5.82$, $P<0.01$].

Adult male rats did not exhibit a sensitized locomotor response if they had been pretreated with cocaine in the home cage (upper graph, Fig. 8). Although a nonsignificant trend was apparent, the distance traveled scores of adult female rats in the Cocaine-Home group did not

differ from females in the Saline-Home group (lower graph, Fig. 8) [Condition main effect, $F_{1,14}=1.17$, $P=0.30$; ^aCondition $\times$ Time interaction, $F_{4,62}=0.76$, $P=0.57$].

Discussion

Adult rats given a single pretreatment administration of cocaine showed context-dependent behavioral sensitization when tested after 24 hr (Fig. 7; see also Weiss et al. 1989; Fontana et al. 1993; Jackson and Nutt 1993; McDougall et al. 2005, 2007). On both the pretreatment and test day, cocaine stimulated greater locomotor activity in adult female rats than male rats (van Haaren and Meyer 1991; Sircar and Kim 1999; McDougall et al. 2007), although male rats appeared to exhibit a more robust sensitized response (see McDougall et al., 2007). Importantly, context-independent sensitization was not evident when adult rats were either pretreated with cocaine and restricted to the home cage (the Cocaine-Home group) or injected with cocaine 30 min after being returned to the home cage from the activity chamber. Preweanling rats responded very differently than adult rats when using the one-trial sensitization procedure, because rats tested on PD 20 exhibited a sensitized locomotor response (measured in activity chambers) regardless of whether the pretreatment injection of cocaine was administered prior to placement in one of the novel chambers (i.e., the activity, large, or small chambers) or 30 min after being returned to the home cage. The lone exception was the Saline-Large group, which showed a nonsignificant enhancement of locomotor activity on the test day (Fig. 4). The reason for this nonsignificant effect is uncertain, especially since preweanling rats in the Cocaine-Home group became sensitized after a single cocaine pretreatment (Fig. 5). Preweanling rats exhibited a similar pattern of behavior if the pretreatment phase was extended to three days (PD 17–PD 19), although the Saline-Large group now showed a sensitized locomotor response. When these results are considered together, it appears that preweanling rats exhibit behavioral sensitization regardless of whether cocaine is paired with the testing environment, explicitly unpaired with the testing environment, or administered in the home cage.

The ontogeny of behavioral sensitization has been studied in detail (for a review, see Tirelli et al. 2003a) and it seems that the sensitized responding of young and adult rats differs in at least three ways. First, the persistence of sensitized responding appears to be substantially reduced in young rats relative to adults (Fujiwara et al. 1987; Kolta et al. 1990; Ujike et al. 1995; McDougall et al. 1999; Zavala et al. 2000). In one of the first studies to examine the ontogeny of behavioral sensitization, Fujiwara et al. (1987) showed that rats pretreated with methamphetamine on PD 17–PD 21 did not exhibit a sensitized locomotor response when tested after a 15-day drug abstinence period. More recently, we reported that young rats would exhibit behavioral sensitization across a 7-day drug abstinence period if a large number of cocaine pretreatments were administered (Zavala et al. 2000; see also Snyder et al. 1998; McDougall et al. 1999). In contrast, adult rats show context-dependent behavioral sensitization even months after the last psychostimulant exposure (Leith and Kuczenski 1982; Robinson et al. 1982). Second, preweanling rats do not typically exhibit an enhanced locomotor response (i.e., conditioned activity) when challenged with saline in the same chamber where they previously received psychostimulant treatment (Wood et al. 1998; McDougall et al. 1999, 2007; Zavala et al. 2000; but see Tirelli and Ferrara 1997). This pattern of results suggests that excitatory conditioning (especially involving psychostimulants) may be weaker during the preweanling period than in adulthood, because even 10 pairings between the environmental context (CS) and cocaine (US) were unable to induce conditioned activity in young rats (Zavala et al. 2000). Third, preweanling rats show context-independent behavioral sensitization after only one pretreatment administration of cocaine, whereas adult rats require multiple psychostimulant exposures before context-independent behavioral sensitization is evident.

The reason for the latter ontogenetic difference is uncertain, but it may be due to age-related changes in associative and/or memory processes. For example, Tirelli et al. (2003b) hypothesized that administering cocaine in a novel environment establishes a “context-dependent memory” that facilitates expression of the sensitized response. If preweanling rats require multiple trials to form a distinct memory of where the psychostimulant was administered, or if this memory is labile, then one-trial sensitization should not be influenced by the context in which the drug was given. A different explanation has been provided by Robinson and colleagues, who proposed that the behavioral sensitization exhibited by adult animals is primarily mediated by nonassociative cellular changes that are modulated by excitatory and inhibitory associative processes (see Anagnostaras et al. 2002). Excitatory conditioning influences behavioral sensitization because Pavlovian associations form between the environmental context (CS) and psychostimulant (US), such that the CS is capable of eliciting enhanced locomotion (CR) (Anagnostaras and Robinson 1996; Tirelli and Terry 1998). Although once thought to fully explain context-dependent behavioral sensitization (e.g., Tilson and Rech 1973) there are numerous studies showing that excitatory conditioning is not a major factor modulating the strength of the sensitized response (for a fuller discussion, see Carey and Gui 1998; Anagnostaras et al. 2002; Tirelli et al. 2003b). Of more importance is inhibitory conditioning, in which the environment is hypothesized to act as an “occasion-setter” (Rescorla et al. 1985; Holland 1992) that prevents the expression of behavioral sensitization in contexts “where the drug is not expected” (i.e., in environments different from where the drug was initially received) (Anagnostaras et al. 2002). For example, sensitized responding is often not exhibited by adult rats and mice that are challenged with a psychostimulant in a novel chamber that is contextually distinct from the chamber in which they were pretreated (Weiss et al. 1989; Anagnostaras and Robinson 1996; Battisti et al. 1999a; Anagnostaras et al. 2002; Wang and Hsiao 2003). In the latter circumstance, the psychostimulant presumably induced the nonassociative cellular changes necessary for behavioral sensitization, but inhibitory conditioning prevented the expression of a sensitized response in the contextually distinct testing environment (Anagnostaras et al. 2002). In terms of the present study, young rats may have shown context-independent behavioral sensitization because inhibitory conditioning mechanisms, especially involving contextual occasion-setters, were not functioning in an adult-like manner during the preweanling period.

An alternative explanation is that the context-independent sensitization of preweanling rats results from stimulus generalization or the associative/perceptual process of “unitization”. It is well established that young rats are more likely to generalize among stimuli than are adults, even if the sensory events or objects involve different sensory modalities (Haroutunian and Campbell 1979; Molina et al. 1986; Chotro and Alonso 1999). Thus, our preweanling rats may have shown context-independent behavioral sensitization because they were unable to differentiate between the various novel environments (activity, large, or small chambers) as well as the home cage. This possibility seems unlikely, however, because (a) 9-day-old rats are able to distinguish between environments containing home and clean bedding (Gregory and Pfaff 1971; Adams et al. 1985; Vorhees et al. 1995) and (b) 10- to 18-day-old rats are readily able to form drug-environment associations during place preference conditioning (i.e., rats can distinguish between a drug- and nondrug-paired compartment) (Pruitt et al. 1995; Bolanos et al. 1996; Randall et al. 1998).

A “unitization” explanation, on the other hand, more readily accounts for the present data and is consistent with the idea that inhibitory occasion-setting mechanisms are not functionally mature in preweanling rats. Unitization is a process largely restricted to early development where young rats treat two distinguishable stimuli as if they were equivalent (i.e., components of a single event or object) because both stimuli have been paired with the same US (Spear et al. 1988; Kraemer et al. 1989; Lariviere et al. 1990; Molina et al. 1991). For example, Molina et al. (1991) showed that the conditioned aversion caused by pairing a black compartment with

foot shock was potentiated if preweanling rats had previously received multiple odor-shock pairings (i.e., the odor and context were treated as a unitary CS). In terms of the present study, preweanling rats may have shown context-independent behavioral sensitization because the different environmental contexts, although discriminable, were treated as equivalent units. Specifically, the two environments where cocaine was experienced (e.g., the home cage and the activity chamber) may have been organized as a single integrated event (i.e., components of a single CS or occasion-setter). Adult rats do not typically organize stimuli in a unitary manner, therefore the home cage and activity chamber should be treated as distinct environments and result in context-dependent behavioral sensitization.

Lastly, Browman et al. (1998a, b) have reported that adult rats are more likely to show context-independent behavioral sensitization if given repeated daily treatments with high doses of cocaine or amphetamine. Thus, it could be argued that the relatively high dose of cocaine (30 mg/kg) administered during the pretreatment phase of the present study may have overwhelmed the associative processes modulating behavioral sensitization (see Browman et al. 1998a, b). This explanation seems unlikely, however, since preweanling and adult rats were injected with the identical dose of cocaine (30 mg/kg) on the pretreatment day, yet only preweanling rats exhibited context-independent behavioral sensitization. It is also noteworthy that context-independent sensitization is not evident if adult rats or mice are injected with even higher doses of cocaine (40 mg/kg, IP) or amphetamine (14 mg/kg, IP) on the single pretreatment day (Weiss et al. 1989; Fontana et al. 1993; Battisti et al. 1999a, 2000).

In conclusion, adult rats do not exhibit one-trial context-independent behavioral sensitization, whereas preweanling rats show context-independent sensitization under a variety of experimental conditions (e.g., when cocaine is administered in the home cage or in novel environments distinct from the test chamber). These results suggest that the nonassociative mechanisms underlying behavioral sensitization are functionally mature in preweanling rats, but the associative processes modulating the strength of the sensitized response do not function in an adult-like manner during the preweanling period. The nature of these associative processes is uncertain, however they may involve age-dependent changes in the way environmental stimuli are organized (unitization vs. nonunitization).

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Fig. 1.

Mean distance traveled scores ($\pm$ SEM) of preweanling rats injected with 30 mg/kg cocaine (filled squares) or saline (open circles) and placed in activity chambers on the three pretreatment days (i.e., PD 17–PD 19). *Significantly different from rats injected with saline on the same time block ($P < 0.05$).

Fig. 2.

Mean distance traveled scores ($\pm$ SEM) of preweanling rats ($n = 8$ per group) given a challenge injection of cocaine (20 mg/kg, IP) prior to placement in activity chambers on PD 20. Rats in the Cocaine-Chamber groups (*filled squares*) had been injected with cocaine (20 mg/kg, IP) before being placed in their respective chambers (activity, large, or small chambers) on PD 17–PD 19, while rats in the Saline-Chamber groups (*filled triangles*) had been injected with cocaine 30 min after being returned to the home cage. The Control groups (*open circles*) were injected with saline at both time points. The inset shows mean distance traveled collapsed across time blocks 1–12. *Significantly different from the control group ($P < 0.05$).

Fig. 3.

Mean distance traveled scores ($\pm$ SEM) of preweanling rats ($n = 8$ per group) given a challenge injection of cocaine (20 mg/kg, IP) prior to placement in the activity chambers on PD 20. On PD 17–PD 19, rats had been restricted to their home cage and injected with either 30 mg/kg cocaine (*filled squares*) or saline (*open circles*). The inset shows mean distance traveled collapsed across time blocks 1–12. *Significantly different from the Saline-Home group ($P < 0.05$).

Fig. 4.

Mean distance traveled scores ($\pm$ SEM) of preweanling rats ($n = 8$ per group) given a challenge injection of cocaine (20 mg/kg, IP) prior to placement in activity chambers on PD 20. Rats in the Cocaine-Chamber groups (*filled squares*) had been injected with cocaine (20 mg/kg, IP) before being placed in their respective chambers (activity, large, or small chambers) on PD 19, while rats in the Saline-Chamber groups (*filled triangles*) had been injected with cocaine 30 min after being returned to the home cage. The Control groups (*open circles*) were injected with saline at both time points. The inset shows mean distance traveled collapsed across time blocks 1–12. *Significantly different from the control group ($P < 0.05$).

Fig. 5.

Mean distance traveled scores ($\pm$ SEM) of preweanling rats ($n = 8$ per group) given a challenge injection of cocaine (20 mg/kg, IP) prior to placement in the activity chambers on PD 20. On PD 19, rats had been restricted to their home cage and injected with either 30 mg/kg cocaine (*filled squares*) or saline (*open circles*). The inset shows mean distance traveled collapsed across time blocks 1–12. *Significantly different from the Saline-Home group ($P < 0.05$).

Fig. 6.

Mean distance traveled scores ($\pm$ SEM) of adult rats injected with 30 mg/kg cocaine or saline and placed in activity chambers on the pretreatment day (PD 79). Cocaine-Female = *filled squares*; Cocaine-Male = *filled circles*; Saline-Female = *open squares*; Saline-Male = *open circles*. *Significantly different from rats injected with saline on the same time block ($P < 0.05$).

Fig. 7.

Mean distance traveled scores ($\pm$ SEM) of adult male and female rats ($n = 8$ per group) given a challenge injection of cocaine (20 mg/kg, IP) prior to placement in the activity chambers on PD 80. On PD 79, rats had been transported to either the activity chambers (upper graph) or restricted to the home cage (lower graph), with drug pretreatments being the same as described in Figs. 4 and 5. The inset shows mean distance traveled collapsed across both sex and time blocks 1–12. *Significantly different from the Activity-Control group ($P < 0.05$).

Fig. 8. Mean distance traveled scores ($\pm$ SEM) of adult male and female rats ($n = 8-10$ per group) given a challenge injection of cocaine (20 mg/kg, IP) prior to placement in the activity chambers on PD 80. On PD 79, rats had been restricted to their home cage and injected with either 30 mg/kg cocaine (*filled squares*) or saline (*open circles*). The inset shows mean distance traveled collapsed across time blocks 1-12.

Table 1
The environments where each preweanling group received injections during the pretreatment phase and the test day

Groups	Pretreatment environments (PD 17–PD 19 or PD 19)				Test day (PD 20)	
	Activity Chamber	Large ¹ Chamber	Small ² Chamber	Tri ³ Chamber	Home Cage	Activity Chamber
Cocaine-Activity	Cocaine				Saline	Cocaine
Saline-Activity	Saline				Cocaine	Cocaine
Activity-Control	Saline				Saline	Cocaine
Cocaine-Large		Cocaine			Saline	Cocaine
Saline-Large		Saline			Cocaine	Cocaine
Large-Control		Saline			Saline	Cocaine
Cocaine-Small			Cocaine		Saline	Cocaine
Saline-Small			Saline		Cocaine	Cocaine
Small-Control			Saline		Saline	Cocaine
Cocaine-Tri ⁴				Cocaine	Saline	Cocaine
Saline-Tri ⁴				Saline	Cocaine	Cocaine
Tri-Control ⁴				Saline	Saline	Cocaine
Saline-Home					Saline	Cocaine
Cocaine-Home					Cocaine	Cocaine

¹ Mouse operant conditioning chambers.
² Small Plexiglas anesthesia chambers.
³ The Tri-Chamber groups were assigned to a different chamber (activity, large, and small) on PD 17–PD 19.
⁴ Tri-chamber groups were not included when drug pretreatment occurred on only PD 19 (Experiment 2).

Table 2

The environments where each adult group received injections on the pretreatment day and the test day

Groups	<u>Pretreatment environment (PD 79)</u>		<u>Test day (PD 80)</u>
	Activity Chamber	Home Cage	Activity Chamber
Cocaine-Activity	Cocaine	Saline	Cocaine
Saline-Activity	Saline	Cocaine	Cocaine
Activity-Control	Saline	Saline	Cocaine
Saline-Home		Saline	Cocaine
Cocaine-Home		Cocaine	Cocaine