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Importance of associative learning processes for the one-trial behavioral sensitization of preweanling rats

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Abstract

During adulthood, associative learning is necessary for the expression of one-trial behavioral sensitization; however, it is uncertain whether the same associative processes are operative during the preweanling period. Two strategies were used to assess the importance of associative learning for the one-trial behavioral sensitization of preweanling rats. In the initial experiments, we varied both the sequence and time interval between presentation of the conditioned stimulus (CS, novel environment) and unconditioned stimulus (US, cocaine). In the final experiment, we determined whether ECS-induced retrograde amnesia would disrupt one-trial behavioral sensitization. Results showed that robust sensitized responding was apparent regardless of the sequence in which cocaine and the novel environment (the presumptive CS) were presented. Varying the time between CS and US presentation (0, 3, or 6 h) was also without effect. Results from Experiment 3 showed that single or multiple ECS treatments did not alter the expression of the sensitized response. Therefore, these data indicated that the one-trial behavioral sensitization of preweanling rats was exclusively mediated by nonassociative mechanisms and that associative processes did not modulate sensitized responding. These findings are in contrast to what is observed during adulthood, because adult rats exhibit one-trial behavioral sensitization only when associative processes are operative.

Keywords

behavioral sensitization; cocaine; electroconvulsive shock; ontogeny; rat

Introduction

Behavioral sensitization occurs when rats repeatedly exposed to a psychostimulant drug (e.g., cocaine) show an augmented behavioral response after a challenge injection with the same drug (Robinson and Becker, 1986; Kalivas and Stewart, 1991). In adult rats and mice, behavioral sensitization is more robust when drug pretreatment and testing occur in the same previously novel environment (Post *et al.*, 1981; Badiani *et al.*, 1997; Carey and Gui, 1998; Tirelli and Terry, 1998; Battisti *et al.*, 2000), however, sensitized responding can occur in situations where the psychostimulant was never associated with the testing environment (Vezina and Stewart, 1990; Browman *et al.*, 1998a, b; Partridge and Schenk, 1999; Battisti *et al.*, 2000). Rather than representing two qualitatively different types of behavioral sensitization (context-specific vs context-independent), it is more likely that sensitization is

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mediated by a common set of nonassociative neural mechanisms that are can be modulated by associative learning (Anagnostaras and Robinson, 1996; Anagnostaras *et al.*, 2002; Wang and Hsiao, 2003).

According to one model, both excitatory and inhibitory conditioning modulates the expression of behavioral sensitization (Anagnostaras and Robinson, 1996; Anagnostaras *et al.*, 2002). Excitatory conditioning influences sensitized responding because an association forms between the environmental context (CS) and the psychostimulant (US), which results in the CS eliciting locomotion (CR). Inhibitory conditioning probably plays a more critical role, because the environment is postulated to act as an “occasion-setter” (see Rescorla *et al.*, 1985; Holland, 1992) that prevents the expression of behavioral sensitization in contexts where the drug was explicitly unpaired (Stewart and Vezina, 1991; Anagnostaras *et al.*, 2002). Direct evidence for the involvement of inhibitory conditioning in behavioral sensitization was provided by a study in which rats received electroconvulsive shock (ECS) or sham-ECS after daily amphetamine administration (Anagnostaras *et al.*, 2002; see also Wang and Hsiao, 2003). ECS-treated rats exhibited a robust sensitized response regardless of whether amphetamine was administered in the same environmental context (i.e., context-specific sensitization) or in a distinctly different environment (i.e., context-independent sensitization). Presumably, context-independent sensitization was evident because retrograde amnesia, a consequence of ECS treatment, disrupted the inhibitory associations modulating the expression of behavioral sensitization (Anagnostaras *et al.*, 2002).

For adult animals, the relative importance of associative factors appears to increase when the drug pretreatment phase consists of a single psychostimulant administration (for discussion, see Pert *et al.*, 1990; White *et al.*, 1998). More specifically, adult rats and mice do not show one-trial behavioral sensitization if drug pretreatment and testing occur in distinctly different environments or if drug pretreatment occurs in the home cage (Drew and Glick, 1989; Weiss *et al.*, 1989; Jackson and Nutt, 1993; Battisti *et al.*, 1999a, b, 2000; McDougall *et al.*, 2007, 2009b). One-trial behavioral sensitization also occurs during early ontogeny; however the pattern of sensitized responding can differ dramatically depending on age of the animal. For example, preweanling rats, unlike adults, show robust context-independent sensitization after a single pretreatment administration of cocaine. That is, rats pretreated with cocaine in the home cage on postnatal day (PD) 19 exhibit a large sensitized response when given a challenge injection of cocaine in an activity chamber on PD 20 or PD 21 (McDougall *et al.*, 2007, 2009a, b; Herbert *et al.*, 2010). The factors responsible for this interesting ontogenetic difference remain uncertain, but it appears that age-dependent changes in the importance of associative processes, or the nature of the drug–environment association itself, may underlie the behavioral differences exhibited by adult and preweanling rats.

In a series of studies we examined the associative and nonassociative factors governing one-trial behavioral sensitization in preweanling rats. To date, we have determined that the context-independent sensitization shown by preweanling rats is not dependent on a high dose of cocaine being administered on the pretreatment day (Herbert *et al.*, 2010), nor is the occurrence of behavioral sensitization paradigm-specific (i.e., context-independent sensitization was evident if young rats were pretreated with cocaine in the home cage or in a novel environment distinct from the activity chambers) (McDougall *et al.*, 2009b; Herbert *et al.*, 2010). Moreover, interoceptive or injection cues cannot account for the one-trial context-independent behavioral sensitization of preweanling rats. Specifically, a robust sensitized response was apparent on the test day even though rats were anesthetized prior to receiving a pretreatment injection of cocaine (Herbert *et al.*, 2010). When results from these various studies are considered together, it appears that associative learning is essential for the one-trial behavioral sensitization of adult rats (i.e., inhibitory conditioning may preclude

the occurrence of context-independent sensitization), while the same associative processes are not operative in preweanling rats.

The purpose of the present study was to further assess the importance of associative processes for the one-trial behavioral sensitization of preweanling rats. In the initial experiments, we varied both the sequence and time interval between CS-US (novel environment-cocaine) presentation. In Experiment 1, a trace conditioning procedure was used in which separate groups of preweanling rats were pretreated with cocaine in a novel activity chamber or 0.5, 3, or 6 h later in the home cage. In Experiment 2, a backward conditioning procedure was used in which cocaine pretreatment (US) occurred either 3 or 6 h before placement in a novel activity chamber (the presumptive CS). For both experiments, sensitized responding was assessed one day later. Our rationale for conducting the first two experiments was that Pavlovian conditioning is typically very sensitive to manipulations affecting CS-US contiguity (for a review, see Mackintosh, 1974); thus increasing the interstimulus interval should have weakened or eliminated any association that might have formed between the novel environment and the psychostimulant. Of course, cocaine must be administered in some location, so those groups not receiving drug in the novel activity chambers were injected with cocaine in the home environment. If an association between the home environment and cocaine formed, it would be expected to disrupt, rather than promote, the expression of behavioral sensitization in the activity chamber. In the third experiment, we used a different strategy and examined whether ECS-induced retrograde amnesia would diminish the strength of the sensitized response. There is a long-established literature showing that ECS disrupts memory for various types of Pavlovian associations (Heistad, 1958; Misanin *et al.*, 1968; Springer, 1975; Quartermain *et al.*, 1988; Shaw, 1988); therefore, we administered ECS after the single CS-US (environment-drug) presentation in order to determine whether a memory-dependent process (i.e., Pavlovian conditioning) is important for the one-trial behavioral sensitization of preweanling rats. Multiple ECS exposures are sometimes necessary to induce memory impairment in adult rats (Andrade *et al.*, 2002; Rao *et al.*, 2002), so ECS was administered either once (Experiment 3a) or three times (Experiment 3b) between drug pretreatment and sensitization testing.

Methods

Subjects

Subjects were 272 male and female rats of Sprague-Dawley descent (Charles River, Hollister, California, USA) that were born and bred at California State University, San Bernardino (CSUSB). Litters were culled to 10 pups on PD 4. Except during testing, rat pups were kept with the dam and littermates in large polycarbonate maternity cages (56 × 34 × 22 cm) with wire lids and Tek-Fresh® bedding (Harlan, Indianapolis, Indiana, USA). Food and water were freely available. The colony room was maintained at 22–24°C and kept under a 12 h light cycle, with lights on at 7:00. Testing was done in a separate experimental room and was conducted during the light phase of the cycle. Subjects were cared for according to the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23, Revised 1996) under a research protocol approved by the Institutional Animal Care and Use Committee of CSUSB.

Apparatus

Behavioral testing was done in commercially available (Coulbourn Instruments, Allentown, Pennsylvania, USA) activity monitoring chambers (25.5 × 25.5 × 41 cm), consisting of acrylic walls, a plastic floor, and an open top. Each chamber included an X–Y photobeam array, with 16 photocells and detectors, that was used to determine distance traveled (locomotor activity). Photobeam resolution was 0.76 cm, with the position of each rat being

determined every 100 ms. ECS was produced by a pulse generator (ECT Unit #57800, Ugo Basile, Collegeville, Pennsylvania, USA).

Procedure

Experiment 1: Simultaneous and trace conditioning procedures—On PD 19, preweanling rats in the Simultaneous groups were taken to the testing room and injected with cocaine (30 mg/kg, i.p.) while being placed in the activity chambers (i.e., cocaine was administered at the same time that rats were exposed to the novel environment). These rats were then returned to the home cage and injected with saline 0.5, 3, or 6 h later. Rats in the Trace groups were injected with saline while being placed in the activity chambers and injected with cocaine (30 mg/kg, IP) 0.5, 3, or 6 h after being returned to the home cage (i.e., cocaine was administered at various time points after rats were exposed to the novel environment). The Acute Control groups were injected with saline at the same time points (i.e., while being placed in the activity chambers and 0.5, 3, or 6 h after being returned to the home cage). For all groups, distance traveled was measured in the activity chambers for 30 min. In all cases, “home” refers to the normal maternity cage that includes both the dam and littermates.

On PD 20 (i.e., 24 h after behavioral testing), all rats ($N = 72$) received a challenge injection of 20 mg/kg cocaine to determine the occurrence of behavioral sensitization. After drug administration, rats were immediately placed in activity chambers where distance traveled was measured for 60 min.

Experiment 2: Simultaneous and backward conditioning procedures—On PD 19, preweanling rats in the Simultaneous groups were taken to the testing room and injected with cocaine (30 mg/kg, IP) while being placed in the activity chambers. Either 3 or 6 h prior to the cocaine injection, rats had been injected with saline in the home cage. Rats in the Backward groups were injected with saline while being placed in the activity chambers. Either 3 or 6 h prior to the saline injection, rats had been injected with cocaine in the home cage (i.e., cocaine was administered at various time points before rats were exposed to the novel environment). The Acute Control groups were injected with saline at the same time points. For all groups, distance traveled was measured in the activity chambers for 30 min.

In addition, separate groups of preweanling rats were restricted to the home cage and never exposed to the activity chambers on PD 19. Rats in the Unpaired 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, with half of the rats in the Unpaired group receiving the saline injection first followed by the cocaine injection. The Acute Control group received saline at both time points.

On PD 20, all rats ($N = 80$) were injected with cocaine (20 mg/kg, i.p.) and immediately placed in activity chambers. Distance traveled was measured for 60 min.

Experiment 3: Effects of ECS on one-trial behavioral sensitization—In Experiment 3a, rats in the Simultaneous groups were taken to the testing room on PD 19 and injected with cocaine (30 mg/kg, i.p.) while being placed in the activity chambers. These rats were then returned to the home cage and injected with saline 0.5 h later. Rats in the Trace groups were injected with saline while being placed in the activity chambers and injected with cocaine (30 mg/kg, IP) 0.5 h after being returned to the home cage. The Acute Control groups were injected with saline at the same time points. For all groups, distance traveled was measured in the activity chambers for 30 min. Groups were further subdivided and rats were given a single sham or ECS administration 1 or 4 h after behavioral testing (i.e., either 0.5 or 3.5 h after receiving their home cage injection). ECS was produced by

administering 0.5 msec electrical pulses (85 mA, 100 Hz) via ear electrodes for a duration of 1 sec. The same ECS parameters were previously used with young rats on PD 28 (Kim *et al.*, 2002). Sham controls were treated in an identical manner, except that no electrical current was administered.

On PD 20 (i.e., 24 h after behavioral testing), all rats ($N = 72$) received a challenge injection of 20 mg/kg cocaine to determine the occurrence of behavioral sensitization. After drug administration, rats were placed in activity chambers where distance traveled was measured for 60 min.

Procedures for Experiment 3b were nearly identical to those just described, with preweanling rats in the Simultaneous, Trace, and Acute Control groups being given a sham or ECS treatment 1 h after behavioral testing on PD 19. On PD 20, no extra drug-environment pairings were provided, but rats were given two additional sham or ECS treatments 24 and 28 h after the initial ECS administration. On PD 21 (i.e., 48 h after behavioral testing), all rats ($N = 48$) received a challenge injection of 20 mg/kg cocaine to determine the occurrence of behavioral sensitization.

Drugs

(–)-Cocaine hydrochloride (Sigma, St. Louis, Missouri, USA) was dissolved in saline and injected i.p. at a volume of 5 ml/kg.

Statistics

For all experiments, omnibus repeated-measures analyses of variance (ANOVAs) were used for the statistical analysis of distance traveled data. Specifically, Group $\times$ Interval Condition $\times$ Time Block ANOVAs were used for analyzing the test day data from Experiments 1 and 2; whereas, Group $\times$ ECS Condition $\times$ Time Block ANOVAs were used for Experiments 3a and 3b. 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 ($P < 0.05$).

Litter effects were controlled through both experimental design and statistical procedures. In most circumstances, no more than one subject per litter was found in a particular group. In situations where this rule was violated (e.g., analyses of the pretreatment day), a single litter mean was calculated from multiple littermates assigned to the same group (Holson and Pearce, 1992; Zorrilla, 1997). In all cases, 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). For each experiment, a nearly equal number of male and female preweanling rats were assigned to each group. Preliminary between-subjects analyses indicated that distance traveled data did not differ according to sex, so this variable was not included in subsequent analyses.

Results

Experiment 1: Simultaneous and trace conditioning procedures

On the pretreatment day (PD 19), rats injected with 30 mg/kg cocaine traveled greater distances than saline controls on all six time blocks (Fig. 1) [Drug main effect, $F(1,7) = 54.26$, $P < 0.001$; Drug $\times$ Time Block interaction, $F(5,35) = 4.54$, $P < 0.01$ and Tukey tests]. On PD 20, behavioral sensitization was evident because rats in the Simultaneous and Trace

groups traveled significantly greater distances than rats given cocaine for the first time on the test day (i.e., the Acute Control group) (Fig. 2) [Group main effect, $F(2,12) = 8.26$, $P < 0.01$ and Tukey tests]. Differences between the Acute Control group and the two cocaine-pretreated groups (i.e., the Simultaneous and Trace groups) were statistically significant on time blocks 2, 3, and 8–12 [$^a\text{Drug} \times \text{Time Block}$ interaction, $F(13,80) = 2.56$, $P < 0.01$ and Tukey tests]. On time block 1, the only significant difference was between the Simultaneous and Acute Control groups [Tukey tests]. Importantly, rats responded similarly regardless of whether the second pretreatment injection was administered 0.5, 3, or 6 h after rats were returned to the home cage (i.e., the main effect and interactions involving interval condition were not statistically significant). In the case of the Trace group, this meant that behavioral sensitization was expressed even though the pretreatment injection of cocaine was administered more than 6 h after placement in the activity chamber.

Experiment 2: Simultaneous and backward conditioning procedures

On the pretreatment day (PD 19), rats injected with 30 mg/kg cocaine immediately before placement in the activity chambers traveled greater distances than saline controls (Fig. 3) [$^a\text{Drug}$ main effect, $F(2,11) = 19.84$, $P < 0.001$ and Tukey tests]. In contrast, distances traveled were not significantly elevated if 30 mg/kg cocaine was administered either 3 or 6 h before behavioral assessment. On the test day (PD 20), rats in the Simultaneous and Backward groups exhibited more locomotor activity than rats in the Acute Control group (Fig. 4) [Group main effect, $F(2,14) = 15.12$, $P < 0.001$ and Tukey tests]. The same pattern of effects was evident regardless of when cocaine was administered on the pretreatment day (i.e., the main effect and interactions involving interval condition were not statistically significant). Thus, rats in the Backward group expressed behavioral sensitization even though they had received cocaine up to 6 h before being placed in the activity chambers on the pretreatment day.

In a separate component of Experiment 2, distances traveled on the test day by the Unpaired group (i.e., rats injected with cocaine and restricted to the home cage on the pretreatment day) were significantly greater than the Acute Control group (Fig. 5) [Group main effect, $F(1,7) = 204.85$, $P < 0.001$]. Thus, sensitized responding occurred even if rats were never exposed to the activity chamber on the pretreatment day.

Experiment 3: Effects of one or three ECS treatments

For Experiments 3a and 3b, distances traveled on the pretreatment day (PD 19) were essentially the same as shown in Fig. 1. Specifically, distances traveled by rats pretreated with 30 mg/kg cocaine ($M = 8,438$ cm, $SEM = 464$) were significantly greater than rats pretreated with saline ($M = 2,960$ cm, $SEM = 147$) [Drug main effect, $F(1,15) = 114.22$, $P < 0.001$].

In Experiment 3a, cocaine-pretreated rats exhibited a sensitized response on the test day (PD 20), because rats in the Simultaneous and Trace groups had traveled greater distances than the Acute Controls (Fig. 6) [Group main effect, $F(2,14) = 5.27$, $P < 0.05$ and Tukey tests]. Administering ECS either 1 or 4 h after behavioral testing on PD 19 did not differentially affect the test day performance of the various drug groups (i.e., the main effect and interactions involving ECS condition were not statistically significant).

Experiment 3b provided essentially the same results as just described (Fig. 7). Relative to the Acute Control group, rats in the Simultaneous and Trace groups exhibited elevated levels of locomotor activity on the test day (PD 21) [Group main effect, $F(2,14) = 8.54$, $P < 0.01$ and Tukey tests]. Administering a series of three ECS treatments on PD 19 and PD 20 did not affect the overall pattern of locomotor activity exhibited by the Simultaneous, Trace,

and Acute Control groups. Thus, increasing the number of ECS treatments to three did not impact the expression of locomotor sensitization. Interestingly, test day distances traveled of rats given three ECS treatments ($M=37,704$ cm, $SEM=2,888$) were significantly elevated when compared to rats given three sham treatments ($M=29,506$ cm, $SEM=2,478$) [Shock main effect, $F(1,7) = 9.16$, $P < 0.05$], but this effect did not vary according to drug pretreatment Group [Shock $\times$ Group interaction, $P = 0.46$; Shock $\times$ Group $\times$ Time Block interaction, $P = 0.95$].

Discussion

During adulthood, associative learning can modulate multi-trial and, especially, one-trial behavioral sensitization (Post *et al.*, 1981; Weiss *et al.*, 1989; Badiani *et al.*, 1997; Carey and Gui, 1998; Tirelli and Terry, 1998; Battisti *et al.*, 2000). Many explanations for this phenomenon have been advanced (e.g., Carey and Gui, 1998; Anagnostaras *et al.*, 2002; Tirelli *et al.*, 2003b), with some models proposing that the psychostimulant serves as a US, while the environmental context acts as a CS or occasion-setter (Pert *et al.*, 1990; Stewart and Vezina, 1991; Anagnostaras *et al.*, 2002; Wang and Hsiao, 2003). Unlike what is observed in adult rats and mice, we have reported that preweanling rats exhibit robust context-independent behavioral sensitization on the test day even if cocaine pretreatment was administered 30 min after removal from the activity chamber (McDougall *et al.*, 2007, 2009a, b; Herbert *et al.*, 2010). The results of Experiment 1 confirm this finding and also show that an equally strong sensitized response is evident if the pretreatment injection of cocaine was administered 3 or 6 h after rats were returned to the home cage (Fig. 2). Pavlovian conditioning typically requires a close temporal relationship between the CS and US (Mackintosh, 1974), so these results strongly suggest that the behavioral sensitization exhibited by preweanling rats is not modulated by associations formed between the novel environment (the presumptive CS) and cocaine (the US). Even so, these data are not a definitive test of the hypothesis because there are a few conditioning procedures, such as taste-aversion learning, in which a strong association will form even though the interval between CS and US presentation is many hours (Smith and Roll, 1967; Revusky and Garcia, 1970).

Results from Experiment 2 further discount an associative explanation, since preweanling rats exhibited one-trial behavioral sensitization when a backward conditioning procedure was used. Specifically, sensitized responding was evident on the test day even though rats were given a pretreatment injection of cocaine (US) up to 6 h before placement in the novel activity chamber (the presumptive CS). That an association formed between cocaine and the novel activity chamber is unlikely, because backward conditioning typically requires that the US be presented at the termination of the CS (McNish *et al.*, 1997; Romaniuk and Williams, 2000; but see Domjan and Gregg, 1977). Under these procedural constraints, both excitatory and inhibitory conditioning have been observed using multi-trial and one-trial backward conditioning procedures (Ayres *et al.*, 1987; Miller *et al.*, 1989; McNish *et al.*, 1997; Romaniuk and Williams, 2000; Delamater *et al.*, 2003; Urushihara, 2004). In this regard, it should be remembered that cocaine, like most drugs, is not a discrete US and it could potentially induce physiological effects that persist for minutes, hours, or even days. Hence, it is possible that the CS and US may overlap even if the drug was given hours earlier. Empirical evidence suggests that such an “overlap” did not occur in Experiment 2, because preweanling rats did not exhibit elevated levels of locomotor activity when pretreated with cocaine either 3 or 6 h earlier (Fig. 3). Moreover, data provided by the Unpaired group directly argue against the proposition that preweanling rats in the backward or trace conditions had formed an association between cocaine and the novel activity chamber (the presumptive CS). Specifically, rats exhibited one-trial behavioral sensitization on PD 20

even though they were pretreated with cocaine in the home cage and were never exposed to the activity chambers on PD 19.

In sum, results from Experiments 1 and 2 indicate that preweanling rats in the Trace, Backward, and Unpaired groups did not form an association between the psychostimulant drug and the novel environmental context on the pretreatment day. Instead, it is possible that preweanling rats in these groups formed an association between the psychostimulant and the home environment. According to Anagnostaras *et al.* (2002) such an association should result in inhibitory conditioning and impede, rather than promote, the expression of behavioral sensitization in the activity chamber (i.e., sensitized responding should not be evident in environmental contexts where the drug was explicitly unpaired). Importantly, the latter explanation may only be applicable to adult animals, because preweanling and adult rats do not perceive the environment in the same way. Adult rats treat multiple CSs as discrete events (Spear and McKenzie, 1994), whereas preweanling rats treat two distinguishable stimuli as if they are equivalent (i.e., components of a single event or object) provided that both stimuli were paired with the same US (Spear *et al.*, 1988; Kraemer *et al.*, 1989; Lariviere *et al.*, 1990; Molina *et al.*, 1991). This process is referred to as “unitization” and may explain why preweanling rats showed a sensitized response even though the pretreatment injection of cocaine was administered in the home cage. Specifically, preweanling rats may have exhibited “context-independent” behavioral sensitization because the two environments where they received cocaine (i.e., the home cage and the activity chamber), although discriminable, were perceived as a unitary CS (in essence, the same environment). If true, excitatory conditioning would potentially enhance the sensitized response, while inhibitory conditioning would not suppress it.

Results from Experiment 3 are relevant to this issue, because the memory for these Pavlovian associations, whether based on “unitization” or not, should be sensitive to ECS-induced retrograde amnesia. Instead, single or multiple ECS treatments were unable to disrupt the one-trial behavioral sensitization of preweanling rats (Fig. 6 and 7). In other words, rats exhibited a robust sensitized response on PD 20 or PD 21 even though the memory trace for the drug-environment association was presumably disrupted by ECS. Although few studies have examined the effects of ECS on preweanling rats, there is substantial evidence that ECS can induce retrograde amnesia in adult rats when assessed on a variety of tasks, including conditioned lick suppression, passive avoidance learning, conditioned taste aversion learning, conditioned emotional response, and spatial memory using either the Morris water maze or the eight arm radial maze (Misanin *et al.*, 1968; Beatty *et al.*, 1985; Quartermain *et al.*, 1988; Shaw, 1988; Andrade *et al.*, 2008; Yao *et al.*, 2010). Among those studies assessing the ontogenetic effects of ECS, it appears that memory impairment is more profound in younger rats than adults (Thompson, 1957). That ECS was without effect in the present study strongly suggests that drug-environment associations do not modify the one-trial behavioral sensitization of preweanling rats. This conclusion is consistent with past developmental studies showing that one-trial behavioral sensitization is not sensitive to manipulations designed to weaken or eliminate environmental, injection, or interoceptive cues (McDougall *et al.* 2009b; Herbert *et al.*, 2010).

Not surprisingly, preweanling rats can show behavioral sensitization when the psychostimulant is administered on multiple pretreatment days (for a review, see Tirelli *et al.*, 2003a). In this circumstance, the sensitized responding of preweanling and adult rats share many of the same characteristics. For example, preweanling rats show only context-specific, but not context-independent, behavioral sensitization when multiple drug pretreatments are provided and testing occurs after an extended drug abstinence period (Zavala *et al.*, 2000; see also Snyder *et al.*, 1998; McDougall *et al.*, 1999). This is not to say

that the multi-trial sensitization of preweanling and adult rats is identical, because the sensitized responding of young rats is generally less robust and persists for a shorter period of time. This ontogenetic difference may be due to nonassociative factors (e.g., maturation of the dopamine or NMDA receptor systems), but it is possible that the weaker sensitization exhibited by preweanling rats is a consequence of age-dependent changes in associative learning. Thus, the ability of preweanling rats to exhibit one-trial context-independent sensitization may result from an associative learning deficit (i.e., an inhibitory conditioning deficit), while age-dependent differences in the persistence and strength of the sensitized response might be due to an inability to establish and maintain drug-environment associations over time. Alternatively, Tirelli *et al.* (2003b) have proposed that the formation of a “context-dependent memory”, rather than associative conditioning, is the mechanism by which environmental cues modify sensitized responding. To accommodate data gained using preweanling rats, it is necessary to assume that nonassociative processes are sufficient to induce one-trial behavioral sensitization and that a “context-dependent memory” did not form after a single exposure to the drug-paired environmental context (i.e., preweanling rats showed equally robust context-specific and context-independent behavioral sensitization). The failure of ECS to alter the sensitized responding of preweanling rats is consistent with this explanation. Specifically, it is possible that ECS did not affect behavioral sensitization because a “context-dependent memory” had not formed after a single drug-environment pairing.

In conclusion, associative learning appears necessary for the one-trial behavioral sensitization of adult rats; whereas, ontogenetic studies indicate that associative conditioning does not impact the one-trial behavioral sensitization of preweanling rats. Specifically, context-independent sensitization is observed in young rats when: (a) ECS is administered after drug pretreatment (Fig. 6 and 7); (b) the pretreatment drug is administered in the home cage or a separate novel chamber (Fig. 2, 4, and 5; see also McDougall *et al.*, 2007, 2009a, b; Herbert *et al.*, 2010); (c) the pretreatment drug is administered 3 or 6 h before or after placement in the novel activity chamber (Fig. 2 and 4); and (d) the pretreatment drug is administered under a state of anesthesia (Herbert *et al.*, 2010). The most parsimonious conclusion is that associative/memory processes do not modulate the one-trial behavioral sensitization of preweanling rats. It is unclear whether this age-dependent difference represents a more general learning/memory deficit characteristic of young rats or if it is unique to drug-environment associations.

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Fig 1.

Mean distance traveled (+SEM) on the pretreatment day (i.e., PD 19) of Experiment 1. Rats ($n = 8$ per group) were injected with saline or 30 mg/kg cocaine prior to placement in the activity chambers. *Significantly different from the saline group when collapsed across time blocks 1–6 (Drug main effect, $P < 0.001$). † Significantly different from the saline group on the same time block (Drug $\times$ Time Block interaction and Tukey tests, $P < 0.05$).

Fig. 2. Mean distance traveled ($\pm$ SEM) of rats ($n = 8$ per group) given a challenge injection of cocaine (20 mg/kg, i.p.) on the test day (PD 20) of Experiment 1. On the pretreatment day (PD 19), rats were injected with cocaine while being placed in the activity chambers (i.e., the Simultaneous groups) or 0.5 h (upper graph), 3 h (middle graph), or 6 h (lower graph) after being returned to the home cage (i.e., the Trace groups). The Acute Controls received two injections of saline. *Significantly different from the Acute Control group when collapsed across time blocks 1–12 (Group main effect and Tukey tests, $P < 0.05$).

Fig 3.

Mean distance traveled (+SEM) on the pretreatment day (i.e., PD 19) of Experiment 2. Rats ($n = 8$ per group) were injected with saline or 30 mg/kg cocaine 0, 3, or 6 h prior to placement in the activity chambers. *Significantly different from the saline group when collapsed across time blocks 1–6 (Drug main effect and Tukey tests, $P < 0.05$).

Fig. 4.

Mean distance traveled ($\pm$ SEM) of rats ($n = 8$ per group) given a challenge injection of cocaine (20 mg/kg, i.p.) on the test day (PD 20) of Experiment 2. On the pretreatment day (PD 19), rats were injected with cocaine 3 h (upper graph) or 6 h (lower graph) before being placed in the activity chamber (i.e., the Backward groups) or while being placed in the activity chambers (i.e., the Simultaneous groups). The Acute Controls received two injections of saline. *Significantly different from the Acute Control group when collapsed across time blocks 1–12 (Group main effect and Tukey tests, $P < 0.05$).

Fig. 5.

Mean distance traveled (+SEM) of rats ($n = 8$ per group) given a challenge injection of cocaine (20 mg/kg, i.p.) on the test day (PD 20) of Experiment 2. On the pretreatment day (PD 19), all rats were restricted to the home cage (i.e., they were never exposed to the activity chamber) and injected with either cocaine (i.e., the Unpaired group) or saline (i.e., the Acute Control group). *Significantly different from the Acute Control group when collapsed across time blocks 1–12 (Group main effect, $P < 0.001$).

Fig. 6.

Mean distance traveled ($\pm$ SEM) of rats ($n = 8$ per group) given a challenge injection of cocaine (20 mg/kg, i.p.) on the test day (PD 20) of Experiment 3a. On the pretreatment day (PD 19), rats received their first injection (either saline or 30 mg/kg cocaine) while being placed in the activity chamber and then received their second injection 0.5 h after being returned to the home cage. Rats received a single sham or ECS treatment 1 or 4 h after completion of behavioral testing on PD 19. *Significantly different from the Acute Control group when collapsed across time blocks 1–12 (Group main effect and Tukey tests, $P < 0.05$).

Fig. 7.

Mean distance traveled ($\pm$ SEM) of rats ($n = 8$ per group) given a challenge injection of cocaine (20 mg/kg, i.p.) on the test day (PD 21) of Experiment 3b. On the pretreatment day (PD 19), rats received their first injection (either saline or 30 mg/kg cocaine) while being placed in the activity chamber and then received their second injection 0.5 h after being returned to the home cage. Rats received three sham (upper graph) or ECS (lower graph) treatments on PD 19 and PD 20 (i.e., 1, 25, and 29 h after completion of behavioral testing).

*Significantly different from the Acute Control group when collapsed across time blocks 1–12 (Group main effect and Tukey tests, $P < 0.05$).