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Ontogeny of one-trial behavioral sensitization in preweanling, adolescent, and adult rats: Differential effects of cocaine and methamphetamine

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ONTOGENY OF ONE-TRIAL BEHAVIORAL SENSITIZATION IN PREWEANLING, ADOLESCENT, AND ADULT RATS: DIFFERENTIAL EFFECTS OF COCAINE AND METHAMPHETAMINE

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ABSTRACT

Preweanling rats exhibit cocaine-induced, but not methamphetamine-induced, behavioral sensitization during the late preweanling period; however, it is uncertain whether this drug-specific effect varies across ontogeny or is unique to a particular developmental period. The purpose of this thesis was to assess the ontogeny of cocaine- and methamphetamine-induced one-trial locomotor sensitization in preweanling, adolescent, and adult rats. In a series of two experiments, rats were pretreated with cocaine (30 mg/kg, IP) or methamphetamine (4 mg/kg, IP) before being placed in a novel activity chamber or the home cage on PD 12, PD 16, PD 20, PD 24, PD 34, or PD 79. Rats were then challenged with the same psychostimulant (20 mg/kg cocaine or 4 mg/kg methamphetamine) on PD 13, PD 21, PD 25, PD 35, or PD 80, with distance traveled being measured for 180 minutes. Cocaine produced locomotor sensitization on PD 13, PD 17, and PD 21; whereas, methamphetamine-induced behavioral sensitization was evident on PD 13 and PD 17. In general, preweanling rats showed robust context-dependent and context-independent behavioral sensitization at all ages, with the exception that cocaine only produced context-specific sensitization at PD 13. In contrast, preadolescent, adolescent, and

adult rats did not exhibit one-trial behavioral sensitization when challenged with cocaine or methamphetamine. Therefore, there are clear ontogenetic changes in the expression of one-trial cocaine- and methamphetamine-induced behavioral sensitization. Regardless of psychostimulant, robust sensitized responding was observed at younger ages but completely disappeared during preadolescence and adolescence. The reason for this variation across ontogeny is uncertain, but it is possible that: (a) pharmacokinetic factors may be responsible for ontogenetic changes in one-trial behavioral sensitization, or (b) neural substrates mediating sensitization may differ across ontogeny. In terms of human relevance, results from my thesis highlight the risks involved in early psychostimulant use and show that mechanisms associated with addiction (i.e., behavioral sensitization) are operating during early ontogeny.

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CHAPTER ONE

HUMAN MODELS OF ADDICTION

Psychostimulants (i.e., cocaine and amphetamine) have long been known to have addictive properties in humans. Although abused throughout human history, psychostimulant use has become an ever-growing problem in society over the past two decades (Sax & Strakowski, 2001). One widely used model to study the underlying mechanisms of drug addiction is behavioral sensitization. Behavioral sensitization is defined as the progressive increase in a behavioral response due to intermittent exposure to a psychostimulant. This increase in drug-induced behavioral effects (i.e., a sensitized response) is probably mediated by the same neural substrates that are responsible for the drug's rewarding actions (Robinson & Berridge, 2001; Sax & Strakowski, 2001; Strakowski & Sax, 1998).

The biological mechanisms underlying behavioral sensitization have been widely studied in rodents, although there is recent evidence that behavioral sensitization also occurs in humans and can be indicative of addictive behavior (Strakowski & Sax, 1998). Despite the lack of human studies on drug-induced behavioral sensitization, Robinson and Berridge (1993) have proposed

that sensitization may underlie the development of drug craving and, in turn, contribute to psychostimulant dependence and relapse. As an example, Strakowski and Sax (1998) assessed behavioral sensitization in humans ranging in age from 18-45, who had no prior diagnoses of psychiatric or substance use disorders and no history of stimulant use. Subjects were administered three oral doses of amphetamine (0.25 mg/kg) or placebo across a six-day span. A progressive increase in eye-blink rate and motor activity was observed following each amphetamine administration. These findings show that behavioral sensitization occurs in humans and suggests that this phenomenon may be an important component of drug addiction.

In humans, environmental context is known to play a critical role in many addiction-related processes, including drug tolerance, withdrawal, and craving (Robinson & Berridge, 1993, 2001; Taylor, Olausson, Quinn, & Torregrossa, 2009). Behavioral sensitization is no exception, because environmental stimuli that become associated with a psychostimulant drug are able to facilitate the sensitization process. For example, animals exhibit a more robust sensitized response when acquisition and testing occur in the same previously novel environment

(Badiani, Camp, & Robinson, 1997; Battisti, Uretsky, & Wallace, 2000; Carey & Gui, 1998; Tirelli & Terry, 1998). Similarly, behavioral sensitization in humans also seems to be influenced by environmental context.

In many ways, behavioral sensitization differs across ontogeny. For example, adult rats show a robust and persistent sensitized response after multiple psychostimulant administrations (Badiani et al., 1997; Battisti et al., 2000; Carey & Gui, 1998; Tirelli & Terry, 1998); whereas preweanling rats exhibit a less robust sensitized response that persists for only about a week (McDougall, Collins, Karper, Watson, & Crawford, 1999; Tirelli, 1997; Zavala, Nazarian, Crawford, & McDougall, 2000). The impact of associative learning processes on behavioral sensitization also differs across ontogeny. This difference is most easily observed when only a single pretreatment administration of a psychostimulant is given. Adult rats, for example, are not capable of exhibiting one-trial context-independent behavioral sensitization. Conversely, preweanling rats readily display a robust context-independent behavioral response after a single pretreatment administration of cocaine (McDougall, Cortez, Palmer, Herbert, Martinez, Charntikov, & Amodeo, 2009a). When considered together, these findings suggest that age

of the animal is a critical factor determining whether contextual cues affect the expression of behavioral sensitization (McDougall, Baella, Stubner, Halladay, & Crawford, 2007). Specifically, the nonassociative and associative properties underlying behavioral sensitization appear to differ across ontogeny.

Therefore, the purpose of this thesis was to examine cocaine- and methamphetamine-induced behavioral sensitization during the early, middle, and late preweanling periods, as well as during preadolescence, adolescence, and adulthood. In addition, the importance of environmental context for behavioral sensitization was assessed in the various age groups.

CHAPTER TWO

ADULT MULTI-TRIAL BEHAVIORAL SENSITIZATION

Behavioral sensitization occurs when an animal is initially exposed to a psychostimulant drug (e.g., cocaine or amphetamine), and is then challenged with the same psychostimulant later. This procedure of repeated exposure to a psychostimulant, followed by a challenge injection of the same drug, produces an augmented locomotor or stereotypic response (Kalivas & Stewart, 1991). Amongst the various paradigms used to assess locomotor sensitization in rodents, the most common is a multi-trial procedure. With a multi-trial sensitization paradigm, rats receive multiple psychostimulant pretreatment injections prior to a challenge injection with a lower dose of the same psychostimulant (Robinson & Becker, 1986). Most researchers administer a psychostimulant once or twice daily for one or two weeks, followed by a challenge injection 7 to 14 days later (Anagnostaras & Robinson, 1996; Doremus-Fitzwater & Spear, 2010). However, some researchers have administered the pretreatment injections for up to nine months, followed by a challenge injection to test the expression of sensitization weeks or months later (Hitzemann, Tseng, Hitzemann, Sampath-Khanna, & Loh,

1977; Kolta, Shreve, De Souza & Uretsky, 1985; Tirelli, Laviola & Adriani, 2003a).

Aside from pretreatment injection frequency, the interval between pretreatment administrations also affects the robustness of the sensitized response. Evidence suggests that intermittent amphetamine administration produces more robust and efficacious behavioral sensitization than more frequent injections (Nielsen & Ellison, 1978; Post, 1980). For example, mice show a progressive increase in locomotor activity when 10 injections of d-amphetamine are administered up to seven days apart. Administering cocaine on an intermittent schedule also causes robust behavioral sensitization in mice (Hirabayashi & Alam, 1981; Hirabayashi, Shibazaki, Izuka & Tadaokoro, 1975; Tadokoro, Hirabayashi & Iizuka, 1978).

Length of drug abstinence affects the strength of the sensitized response. Rats injected with amphetamine twice daily for 5 or 15 days needed to be abstinent for more than one day to show behavioral sensitization (Kolta et al., 1985). Moreover, some rats show a much greater sensitized response when the drug abstinence period lasts for 15 or 30 days after repeated amphetamine treatment as opposed to only 3 days (Hitzemann et al., 1977; Kolta et

al., 1985). Post (1980) emphasized the importance of allowing change to develop in the nervous system between drug treatments and challenge injections. Thus, suggesting that a close temporal contiguity of drug injections, especially at high doses, will have the same effect as continuous administration and cause tolerance instead of sensitization (Antelman & Chiodo, 1981; Post 1980, 1981).

Drug dose also plays a critical role in the behavioral effects produced by psychostimulants. For example, a number of studies have shown that repeated administration of amphetamine will enhance locomotor activity at various doses, ranging from <1.0 mg/kg to 10 mg/kg (Robinson, 1984; Short & Shuster, 1976). Typically, repeated administration of low doses of amphetamine cause a progressive enhancement of locomotor activity throughout the duration of the drug's effect. In one case, Segal and Mandell (1974) observed a progressive increase in locomotor activity for 36 days with repeated administration of 0.5 mg/kg amphetamine. In contrast, moderate to high doses of amphetamine cause an initial increase in locomotor hyperactivity, followed by an increase in stereotyped behaviors and a concomitant decrease in locomotion. A subsequent increase in post-stereotypy locomotor activity is often evident (Leith

& Kuczenski, 1982; Robinson & Becker, 1986). This pattern of sensitized locomotor activity, represented by a U-shaped curve, is frequently observed in animals receiving repeated administration of 2.5-7.5 mg/kg amphetamine.

Repeated administration of cocaine also causes a progressive increase in behavioral responsivity (Borowsky & Kuhn, 1991; Post & Rose, 1976). Generally, repeated administration of low (e.g., 5-10 mg/kg) to high doses (e.g., 30-40 mg/kg) of cocaine causes a dose-dependent enhancement in locomotor activity and stereotypy. For example, Borowsky and Kuhn (1991) observed a marked increase in the locomotor response to cocaine after 3 or 7 days of twice-daily cocaine (15 mg/kg) administrations. Kalivas and colleagues also observed a progressive enhancement in the locomotor activity and stereotyped behaviors of rats pretreated with 7.5, 15 or 30 mg/kg cocaine (3 times daily) and challenged with 15 mg/kg cocaine. Although all pretreatment doses of cocaine produced an enhanced behavioral response, the highest pretreatment dose of cocaine (i.e., 30 mg/kg) produced a stronger sensitized locomotor response and prolonged stereotypic behavior (Kalivas, Duffy, Dumars, & Skinner, 1988).

Repeated administration of various doses of methamphetamine also differentially affects behavioral sensitization in rodents. Low to moderate doses of methamphetamine induce dose-dependent increases in locomotor activity that is characterized by high peak activity with a prolonged duration. In contrast, high doses of methamphetamine produce a biphasic pattern of behavioral effects, where animals initially show increased locomotor activity, followed by both a decline in locomotion and an enhancement of stereotyped behavior, and finally post-stereotypy locomotor activity (Hirabayashi & Alam, 1981; Kuribara & Uchihashi, 1994). Consistent with these findings, Hirabayashi and Alam (1981) reported a progressive enhancement of locomotor activity in mice receiving 10 dosings of 1, 2, 4 or 8 mg/kg methamphetamine administered on a 1, 3 to 4, or 7 day interval schedule. The strength of the sensitized response was dependent on dose treatment schedule. Enhanced locomotor activity was typically observed after daily administration of lower doses of methamphetamine (e.g., 1 mg/kg). Moderate doses (e.g., 4 mg/kg) produced more prominent locomotor activity when a 3 to 4 or 7-day drug treatment schedule was used. In general, administration of higher doses of methamphetamine (e.g., 8 mg/kg) caused an initial increase

in locomotor activity, followed by a phase of increased stereotyped behavior, and finally a second phase of increased locomotor activity with attenuated stereotyped behavior (Hirabayashi & Alam, 1981).

Environmental context plays an important role in the induction and expression of behavioral sensitization of adult rats and mice. Behavioral sensitization is context-dependent if a sensitized response is only apparent when the pretreatment and challenge injection of the psychostimulant are administered in the same previously novel environment (Badiani et al., 1997; McDougall et al., 2009a). Context-independent sensitization is evident when the pretreatment and challenge injection of the psychostimulant are administered in different environments (Badiani et al., 1997; McDougall et al., 2009a). That being said, adult animals show a more robust sensitized response under context-dependent conditions, where drug pretreatment and testing occur in the same previously novel environment (Badiani et al., 1997; Battisti et al., 2000; Carey & Gui, 1998; Tirelli & Terry, 1998).

Anagnostaras and Robinson (1996) found that in certain circumstances, expression of sensitization can be completely context-specific. Drug dose plays an important

role in determining whether context-independent sensitization will be expressed. Repeated treatment with moderate to high doses of psychostimulants can cause an approximately fourfold increase in the strength of the sensitized response (Anagnostaras & Robinson, 1996). At lower doses, however, context-independent sensitization is often not evident, while context-specific sensitization remains strong (Drew & Glick, 1988; Mazurski & Beninger, 1987; Post & Weiss, 1988; Stewart & Vezina, 1991). These studies suggest that regardless of psychostimulant dose, a robust sensitized response can be observed when rats and mice are pretreated and tested in the same environmental context (Badiani et al., 1997; Battisti et al., 2000).

CHAPTER THREE

ADULT ONE-TRIAL BEHAVIORAL SENSITIZATION

Behavioral sensitization is commonly measured using a multi-treatment paradigm; however, enhanced behavioral responses are also observed after a single pretreatment injection of a psychostimulant (Weiss, Post, Pert, Woodward, & Murman, 1989; McDougall, Reichel, Cyr, Karper, Nazarian, & Crawford, 2005). With this "one-trial" paradigm, sensitized responding is typically assessed in the same previously novel environment (e.g., an activity chamber), with a challenge injection of the same psychostimulant being given 24 to 48 hours after the pretreatment injection (Weiss et al., 1989; McDougall et al., 2007). For example, McDougall and colleagues observed robust locomotor sensitization in rats 24 hours after a single pretreatment injection of cocaine (McDougall et al., 2007).

Although sensitized responding is typically assessed 24 to 48 hours after a single pretreatment injection of a psychostimulant, behavioral sensitization has also been exhibited 3-4 weeks later (Robinson, Becker, & Presty, 1982). Specifically, Robinson and colleagues observed enhanced rotational behavior when rats were challenged

with an injection of amphetamine 3-4 weeks after a single injection of a low dose of amphetamine (Robinson et al., 1982). The sensitization-inducing effects of psychostimulants are dose-dependent. Enhanced locomotor activity and stereotypic behavior are typically observed when rats and mice are given a single high-dose injection of a psychostimulant, followed by a low-dose challenge injection of the same psychostimulant 24 to 48 hours later (Battisti, Uretsky, & Wallace, 1999; 2000; Jackson & Nutt, 1992; Weiss et al., 1989). For example, Weiss and colleagues observed increased locomotor activity after pretreating rats with a single high dose of cocaine (i.e., 40 mg/kg) and challenging the same rats with a low dose of cocaine (i.e., 10 mg/kg) 24 hours later (Weiss et al., 1989). Battisti et al. (1999, 2000) also reported a sensitized response in mice challenged with 7.5 mg/kg amphetamine after pretreatment with a single high dose (i.e., 14 mg/kg) of the same drug.

The one-trial procedure typically produces a robust context-dependent sensitized response in adult animals (Battisti et al., 2000; McDougall et al., 2007, Weiss et al., 1989). In contrast, context-independent sensitization is not observed when adult animals are tested in a novel environment 24 to 48 hours after a single pretreatment

injection of a psychostimulant (Battisti et al., 2000; McDougall et al., 2007, 2009a). For example, Battisti et al. (1999) pretreated mice with a single high dose of amphetamine (i.e., 14 mg/kg) before placing them in chambers differing in size, color, and texture of bedding. When the same mice were challenged with a lower dose of amphetamine (7 mg/kg) 24 hours later, only mice pretreated and tested in the same, or nearly identical environments, exhibited a sensitized stereotypic response.

Using a slightly different approach, Weiss et al. (1989) administered either a high dose of cocaine (40 mg/kg) or saline prior to placing rats in the activity chambers for 30 minutes on the pretreatment day. Following the 30 minute session, the same rats were given a second injection of either cocaine or saline in one of several environments: the rats that received cocaine in the activity chambers were given saline and returned to the home cage; the remaining three groups received saline in the activity chambers and were then administered cocaine in the home cage, a Plexiglas cage containing sawdust (as in the activity chambers), or a small wire cage containing no sawdust (dissimilar to the activity chambers). When all groups of rats were challenged with a low dose of cocaine (10 mg/kg) in the same activity chamber on the subsequent

day, a sensitized locomotor response was only evident in rats pretreated and tested in the same, or a nearly identical, environment. Therefore, it appears that, regardless of experimental methodology, adult rats and mice do not exhibit psychostimulant induced one-trial behavioral sensitization when pretreatment and testing occur in distinctly different environments (Battisti et al., 1999; Jackson & Nutt, 1992; McDougall et al., 2007; Weiss et al., 1989).

CHAPTER FOUR

THEORETICAL BASIS FOR CONTEXT-SPECIFIC BEHAVIORAL SENSITIZATION IN ADULT RATS

Despite an extensive literature, the mechanisms underlying psychostimulant-induced behavioral sensitization are not fully understood. According to one view, behavioral sensitization is the direct outcome of a psychostimulant acting on certain neural mechanisms (Kuczenski, Segal, Weinberger, & Browne, 1982; Robinson & Becker, 1986). This is a *nonassociative view* in which conditioned stimuli (CS) (e.g., the environmental context) have minimal or no influence on the induction and expression of behavioral sensitization. Thus, sensitization is a progressive increase in the unconditioned response (UR) to a psychostimulant, which is facilitated by drug-induced changes in neuronal mechanisms that mediate the UR (Anagnostaras & Robinson, 1996).

According to a second view, nonassociative psychostimulant-induced neural changes are a necessary component of behavioral sensitization, but associative processes modulate the development and later expression of the sensitized response (Anagnostaras & Robinson, 1996; Anagnostaras, Schallert, & Robinson, 2002; Wang & Hsiao,

2003). Thus, sensitization is modulated by *associative learning* processes that underlie drug-environment conditioning (Hinson & Poulos, 1981; Siegel, Krank, & Hinson, 1987; Tilson & Rech, 1973). Behavioral sensitization is typically more robust when a psychostimulant drug (i.e., US) is paired with a novel environment (i.e., CS). As a consequence of these pairings, the environmental context is then capable of producing drug-like psychomotor responses (Beninger & Hahn, 1983; Carey, 1986, Fontana, Post, & Pert, 1993; Post, Lockfield, Squillace, & Contel, 1981; Tirelli & Terry, 1998). Two Pavlovian mechanisms are necessary to explain context-specific behavioral sensitization; excitatory conditioning and inhibitory conditioning.

Excitatory conditioning is partially responsible for the increased behavioral response produced by repeatedly administering a drug in the same previously novel environment. Specifically, the unique environmental context acts as the CS and the drug acts as the US. As a result, the conditioned response (CR) produced by the environmental context is similar to the UR produced by the drug (Anagnostaras & Robinson, 1996; Hinson & Poulos, 1981; Siegel et al., 1987; Tilson & Rech, 1973). This environment-drug (CS-US) pairing allows the CS to produce

psychostimulant-like effects when presented alone. Therefore, context-specific sensitization results, in part, from the CR augmenting the natural UR produced by the drug (Anagnostaras & Robinson, 1996). Excitatory conditioning, however, cannot fully explain behavioral sensitization, because sensitized responding is also observed in non-drug paired contexts. Specifically, behavioral sensitization is evident when the rat is tested in an environment not previously paired with a psychostimulant (i.e., in the absence of a CR) (Anagnostaras & Robinson, 1996).

According to classic learning theory, the CR should persist for as long as the sensitized response (Tirelli, Michel, & Brabont, 2005). In fact, Tirelli and colleagues (2005) report that mice will exhibit a CR for much longer than the sensitized response. An excitatory conditioning explanation also predicts that the temporal relationship of the environment-drug pairing plays an important role in producing CRs. In other words, increasing the time interval between exposure to the test environment (i.e., the CS) and drug administration (i.e., the US) should prevent the induction of CRs. Rats, however, are still able to exhibit behavioral sensitization with a long CS-US interval (Crombag, Badiani, Chan, Dell-Orco, Dineen, &

Robinson, 2001). Lastly, if excitatory conditioning is sufficient to explain context-specific sensitization then the conditioned response should extinguish if the rat is exposed to the testing environment in the absence of the psychostimulant. Instead, the amphetamine-induced sensitized response is little affected by pre-exposing the animal to the context without the psychostimulant (Anagnostaras & Robinson, 1996; Battisti et al., 2000; Carey & Gui, 1998; Jodogne, Marinelli, Le Moal, & Piazza, 1994; Stewart & Vezina, 1991). For these various reasons, excitatory conditioning by itself is insufficient to explain context-specific behavioral sensitization.

Inhibitory conditioning, on the other hand, may be the more critical associative process modulating behavioral sensitization (Anagnostaras et al., 2002; Stewart & Vezina, 1988; Wang & Hsiao, 2003). When the context (i.e., CS) and drug (i.e., US) are explicitly paired, inhibitory conditioning does not affect expression of the sensitized response as long as testing occurs in the previously drug-paired chamber. When the context (i.e., CS) and drug (i.e., US) are explicitly unpaired, however, the likelihood of a sensitized response occurring decreases due to negative contingent properties brought about by inhibitory conditioning. Under these

circumstances, the contextual stimuli act as an inhibitory CS (CS^-), actively inhibiting the sensitized effects of the drug (UR) (Anagnostaras & Robinson, 1996).

In conclusion, various researchers have proposed that behavioral sensitization is mediated by nonassociative neuroadaptations that are modulated by excitatory and inhibitory conditioning (Anagnostaras & Robinson, 1996; Wang & Hsiao, 2003). Excitatory conditioning, which is less important, increases the strength of the sensitized response; whereas, inhibitory conditioning, which is more important, decreases the strength of the sensitized response in contexts that were unpaired with the drug. In a reformulation of this idea, Anagnostaras et al. (2002) suggest that inhibitory conditioning may rely on an occasion-setting mechanism. "Occasion-setters" are stimuli that not only act as a CS, but also have the ability to modulate responses to other stimuli (Holland, 1985, 1989, 1992; Rescorla, 1985). Thus, a drug-paired context may act as an occasion setter, modulating the expression of the sensitized response (Anagnostaras & Robinson, 1996). That being said, it is important to realize that circumstances inducing and reducing occasion-setters and excitatory CSs are fundamentally different (Rescorla, 1985). For example, it is possible to determine if a stimulus acts as an

occasion-setter or conditioned excitor based on how closely the cue and US are associated (Holland, 1986). Specifically, the induction of occasion-setters is ideal in situations where the nature of the temporal relationship between the contextual stimuli and the USs are more distinct (Bouton, 1993; Bouton & Swartzentruber, 1986; Holland, 1992; Rescorla, Durlach, & Grau, 1985), in which case occasion-setters modulate the excitatory strength of other stimuli (Rescorla, 1985). In terms of sensitization, Anagnostaras et al. (2002) believe that if rats receive a drug in a distinct environment, they form an expectation that the drug will be received in that specific environment. This expectation (i.e., the forming of a distinct CS-US association) may act as an occasion-setter and attenuate the sensitized behavioral response.

Of course, context-independent sensitization is frequently reported (Browman, Badiani, & Robinson, 1998b; Partridge & Schenk, 1999; Vezina & Stewart, 1990). In this circumstance, the strength of the nonassociative neuroadaptations are apparently sufficient to negate the effects of inhibitory conditioning. Specifically, high doses of cocaine and amphetamine have been shown to induce behavioral sensitization regardless of the environmental

context in which the drug is administered (Browman et al., 1998a, 1998b). These findings suggest that the magnitude of the neuroadapations underlying behavioral sensitization may overwhelm associative processes involving inhibitory conditioning. The latter explanation only applies to multi-trial behavioral sensitization, however, because adult rats and mice never exhibit one-trial context-independent sensitization regardless of the dose of psychostimulant used (McDougall et al., 2007; Battisti et al., 2000; Weiss et al., 1989). These findings suggest that, when using a one-trial procedure, the psychostimulant-induced neuroadaptations are never sufficient to overcome the effects of inhibitory conditioning. Only with multiple psychostimulant exposures, often involving high doses of the drug, will the nonassociative neuroadaptations overcome the effects of inhibitory conditioning.

Recently, Tirelli and colleagues have developed a different model to explain context-specific behavioral sensitization (Tirelli, Tambour, & Michel, 2003b). They believe that nonassociative cognitive processes underlie context-specific sensitization. Specifically, the retrieval of information is augmented when the psychostimulant is repeatedly administered (acquisition

and testing) in the same environment. This procedure causes the rat to better integrate contextual cues in memory, thus allowing the expression of a sensitized response (Tirelli et al., 2003b). The drug, therefore, becomes part of the sensitized response by being integrated with the memories of the environmental context. Conversely, if an animal is tested in an environment where contextual memories of the drug-paired environment are absent, a robust sensitized response will not be exhibited due to an impediment of information recall (Tirelli et al., 2003b).

CHAPTER FIVE

MULTI-TRIAL SENSITIZATION IN YOUNG ANIMALS

Like adult rats, preweanling rats exposed to repeated administrations of psychostimulants (e.g., cocaine, amphetamine, and methylphenidate) also exhibit behavioral sensitization (McDougall, Duke, Bolanos, & Crawford, 1994, 1999; Snyder, Katovic, & Spear, 1998). Despite qualitative similarities, the behavioral sensitization exhibited by preweanling and adult rats differ in several ways, including persistence, robustness of the sensitized response, and the role of contextual stimuli.

In contrast to adult rats, which exhibit context-dependent sensitization months after the last psychostimulant exposure, young rats show reduced persistence of sensitized responding (Fujiwara, Kazahaya, Nakashima, Sato, & Otuski, 1987; Kolta, Scalzo, Ali, & Holson, 1990; McDougall et al., 1999; Zavala et al., 2000). In one of the first studies examining the ontogeny of behavioral sensitization, Fujiwara et al. (1987) reported the absence of a sensitized locomotor response in young rats pretreated with methamphetamine for five consecutive days (i.e., PD 17-21) and tested after a 15-day drug abstinence period. Consistent with these

findings, McDougall et al. (1999) observed "short-term" behavioral sensitization after a 5-day pretreatment period; however, when tested seven days after the last pretreatment injection, "long-term" behavioral sensitization was not exhibited by preweanling rats.

Although long-term behavioral sensitization is generally not evident in preweanling rats, persistence of the sensitized response can be enhanced by providing a large number of pretreatment psychostimulant administrations (McDougall et al., 1999; Synder et al., 1998; Zavala et al., 2000). Specifically, Zavala et al. (2000) reported that locomotor sensitization could be observed across a 7-day drug abstinence period if young rats were provided with 10 pretreatment administrations of cocaine. There have been instances where drug dose also plays an important role in the persistence of sensitized responding. For example, behavioral sensitization was evident in preweanling rats after seven abstinence days if high doses of methylphendiate were given during both pretreatment and testing (McDougall et al., 1999). As stated before, sensitized responding often becomes more robust in adult rats as the time between the pretreatment phase and testing is extended (Nielsen & Ellison, 1978; Post, 1980). In young rats, however, the strength of the

sensitized response typically gets less robust across days (Fujiwara et al., 1987; McDougall et al., 1999). Using a different methodology, Smith and Morrell (2008) compared the robustness of psychostimulants in preweanling and adult rats by administering three once-daily cocaine treatments. Preweanling rats (i.e., PD 22) exhibited an initial increase in locomotor activity lasting 30 minutes, followed by a "tolerance-like response" for the remainder of the 3-hour testing session (Smith & Morell, 2008). In contrast, adult rats (i.e., PD 60) exhibited an increase in cocaine-induced locomotor activity across the entire 3-hour testing period (Smith & Morrell 2008).

Although context-specific behavioral sensitization is evident by the first to third weeks of life, drug-context associations appear to strengthen as the animal matures (Tirelli, 2001; Tirelli & Ferrara, 1997; Tirelli et al., 2003a). That being said, drug-context associations last no longer than a week in preweanling rats, but can last many months in adults (Tirelli et al., 2003a). Aside from showing context-dependent behavioral sensitization at a fairly young age, preweanling rats are also capable of exhibiting context-independent behavioral sensitization soon after birth (McDougall et al., 2007, 2009a; Zavala et al., 2000). When preweanling rats (PD 11-20) were

pretreated with cocaine for 5 or 10 days in either activity chambers or home cage, context-independent locomotor sensitization was observed after a 1-day drug abstinence period (McDougall et al., 2007, 2009; Zavala et al., 2000). Interestingly, young rats tested after a 7-day drug abstinence period only showed context-dependent locomotor sensitization (Zavala et al., 2000). Thus, the length of the drug abstinence period seems to increase the importance of environmental factors for the induction of locomotor sensitization in young rats (Zavala et al., 2000).

As mentioned in Chapter 4, "context-dependent" and "context-independent" behavioral sensitization is believed to share a common set of neural mechanisms. Specifically, enhanced behavioral responsiveness is presumably due to non-associative cellular changes, while associative processes play an important role in modulating the sensitized response (Anagnostaras et al., 2002; Stewart & Vezina, 1988). The ability of preweanling rats to exhibit both context-dependent and context-independent behavioral sensitization suggests that the necessary non-associative cellular changes occur after psychostimulant treatment, whereas associative processes are unable to modify the

sensitized response except with a large number of drug-environment pairings (McDougall et al., 2007).

In contrast to adult and preweanling rats, adolescent rats have been reported to show a markedly different response to psychostimulant treatment (Collins & Izenwasser, 2002; Spear, 2000). For example, Collins and Izenwasser (2002) reported the absence of a sensitized response in adolescent rats pretreated with cocaine for seven consecutive days (i.e., PD 28-35) and tested after a 10-day drug abstinence period. Consistent with these findings, Spear and Brick (1979) assessed cocaine-induced behavioral responses on PD 7, PD 14, PD 21, PD 28, and PD 35, and found that rats older than PD 21 did not show elevated locomotor activity. The adolescent period in the rat is often characterized by alternations in novelty seeking and changes in behavioral responsiveness to drugs of abuse. Thus, these age-dependent differences in locomotor activity may be reflective of alterations in the reward value of psychostimulants (Adriani, Chiarotti, & Laviola, 1998; Spear, 2000).

CHAPTER SIX

ONE-TRIAL BEHAVIORAL SENSITIZATION IN YOUNG ANIMALS

In adult animals, the importance of environmental conditioning factors are increased with the "one-trial" sensitization paradigm (White, Joshi, Koeltzow, & Hu, 1998), because adult rats and mice only exhibit a sensitized response when pretreated and challenged with a psychostimulant in the same novel environmental context (Battisti et al., 2000; Jackson & Nutt, 1993; McDougall et al., 2005; Weiss et al., 1989). In contrast, preweanling rats show a different pattern of sensitized responding when using the one-trial paradigm. Specifically, preweanling rats exhibit a robust context-independent sensitized response using various one-trial experimental procedures (McDougall et al., 2007, 2009a). For these reasons, one-trial sensitization has been used as a model to examine ontogenetic differences in behavioral sensitization (McDougall et al., 2007).

In order to determine whether environmental factors modulate one-trial behavioral sensitization in preweanling rats, McDougall and colleagues pretreated one group of PD 19 rats with cocaine and another group of PD 19 rats

with saline and placed them in activity chambers for 30 minutes. These rats were then returned to the home cage and administered either cocaine (if they received saline in the activity chamber) or saline (if they received cocaine in the activity chamber). Preweanling rats exhibited both context-specific and context-independent behavioral sensitization when tested with cocaine on the subsequent day (McDougall et al., 2009a). In a separate experiment, McDougall and colleagues pretreated preweanling rats (PD 19) with either cocaine or saline and restricted them to the home cage. Cocaine-pretreated rats showed context-independent locomotor sensitization when given a challenge injection of cocaine in the activity chambers on the following day (McDougall et al., 2009a). These findings further indicate that the nonassociative neural mechanisms underlying behavioral sensitization are functionally mature in young animals; however, the results also suggest that associative properties modulating the strength of the sensitized response do not function in an adult-like manner (McDougall et al., 2009a). Although speculative, preweanling rats may exhibit context-independent sensitization because inhibitory associative processes are unable to attenuate the

expression of behavioral sensitization in environmental contexts not previously paired with the drug.

Because drug dose is an important factor determining whether adult rats exhibit context-independent behavioral sensitization (Browman et al., 1998a, 1998b), Herbert and colleagues assessed whether varying the pretreatment dose of cocaine would differentially affect the context-specific and context-independent sensitization of preweanling rats (Herbert, Der-Ghazarian, Palmer, & McDougall, 2010). Because adult rats are more likely to exhibit context-independent sensitization if high doses of a psychostimulant are used during the drug pretreatment phase (Browman et al., 1998a, 1998b), it was hypothesized that the context-independent behavioral sensitization of preweanling rats would also be affected by the dose of cocaine used. Herbert and colleagues pretreated one set of PD 19 rats with 10, 20, 30, or 40 mg/kg cocaine, whereas another set of PD 19 rats was pretreated with saline before being placed in activity chambers for 30 minutes. Rats that received saline in the activity chambers were then administered 10, 20, 30, or 40 mg/kg cocaine after being returned to the home cage, while rats that received cocaine in the activity chamber were injected with saline. Regardless of pretreatment dose, context-specific and

context-independent sensitization were exhibited by all rats, thus showing that varying the pretreatment dose of cocaine did not dissociate the context-specific and context-independent sensitization of preweanling rats (Herbert et al., 2010).

Unlike adult rats, the ability of preweanling rats to readily exhibit one-trial context-independent sensitization could indicate that either one-trial behavioral sensitization is not modulated by associative properties during the preweanling period or environmental-drug (CS-US) pairings are processed differently in preweanling and adults rats (McDougall, Kozanian, Greenfield, Horn, Gutierrez, Mohd-Yusof, & Castellanos, 2011a). Consistent with the latter suggestion, adult rats are able to dissociate multiple CSs during environment-drug pairings (Spear & McKenzie, 1994), whereas preweanling rats often treat discrete stimuli as equivalent if they are paired with the same US (e.g., a psychostimulant drug) (Lariviere, Chen, & Spear, 1990; Molina, Hoffman, Serwatka, & Spear, 1991; Spear, Kramer, Molina, & Smoller, 1988). The tendency of young rats to perceive multiple stimuli as a unified CS is referred to as "unitization". This phenomenon could impact behavioral sensitization, because preweanling rats may perceive

multiple environmental contexts as a unified CS if one of the environments had previously been paired with a drug. Specifically, administering cocaine in the home cage on the pretreatment day may have the same associative effects as administering cocaine in the activity chamber (McDougall et al., 2009a, 2011a). According to this explanation, preweanling rats show context-independent behavioral sensitization because they are unitizing the pretreatment and test environment due to the fact that both contexts were paired with a common drug (US) (Spear et al., 1988).

To further examine the factors involved in one-trial behavioral sensitization, McDougall et al. (2011a) assessed whether other psychostimulant drug (aside from cocaine), such as methamphetamine, methylphenidate, or amphetamine, are capable of inducing context-specific and context-independent one-trial behavioral sensitization in preweanling rats. In a series of four experiments, McDougall and colleagues pretreated PD 19 rats with cocaine (30 mg/kg), methamphetamine (2-12 mg/kg), methylphenidate (5-20 mg/kg), or amphetamine (5 mg/kg) before placing them in the activity chamber or home cage. Two days later (PD 21) rats were challenged with the same psychostimulant (20 mg/kg cocaine, 1-8 mg/kg

methamphetamine, 2.5-7.5 mg/kg methylphenidate, or 1-2 mg/kg amphetamine) before being placed in the activity chambers for three hours. Surprisingly, only cocaine, but not various dose combinations of the other psychostimulants, produced one-trial behavioral sensitization in preweanling rats (McDougall et al., 2011a).

Various explanations could account for why only cocaine, but not methamphetamine, methylphenidate, or amphetamine, were able to produce one-trial behavioral sensitization in preweanling rats. Cocaine-induced behavioral sensitization may be mediated by neural mechanisms that are capable of becoming sensitized after a single pretreatment trial, while methamphetamine-, methylphenidate-, and amphetamine-induced behavioral sensitization may rely on different neural pathways. Although this issue has not been extensively studied in the literature, there is evidence that the neural mechanisms mediating amphetamine- and cocaine-induced behavioral sensitization are not identical (Vanderschuren & Kalivas, 2000). Alternatively, the pharmacodynamics of cocaine may make this drug uniquely able to support environment-drug (CS-US) associations (McDougall et al., 2011a). For example, cocaine penetrates the brain quickly

and has a shorter half-life than other psychostimulants (Benuck, Lajtha, & Reith, 1987; Brien, Kitney, Peachey, & Rogers, 1978; Gerasimov, Franceschi, Volkow, Gifford, Gatley, Marsteller, Molina, & Dewey, 2000; Lal & Feldmuller, 1975). In terms of unitization, it is possible that cocaine is more "conditionable" than other psychostimulants; thus, a single exposure to cocaine may be sufficient to produce a drug-environment association. In contrast, methamphetamine, methylphenidate, or amphetamine may require multiple pairings before a drug-environment association is formed (McDougall et al., 2011a).

CHAPTER SEVEN

SUMMARY

In the "one-trial" behavioral sensitization paradigm rats are given a single pretreatment injection of a psychostimulant followed, 24 to 48 hours later, by a challenge injection of the same psychostimulant. Adult rats only exhibit a sensitized response when psychostimulant pretreatment and testing occur in the same novel environmental context (Battisti et al., 2000; Jackson & Nutt, 1993; McDougall et al., 2005; Weiss et al., 1989). In contrast, preweanling rats show robust one-trial context-independent behavioral sensitization when tested in the identical circumstance (Herbert et al., 2010; McDougall et al., 2007, 2009a). The importance of environmental conditioning factors, therefore, are increased when adult rats are tested using the one-trial paradigm (White et al., 1998), while environmental conditioning factors may be unimportant for preweanling rats (McDougall et al., 2011b).

These findings suggest that the underlying neural mechanisms mediating the expression of behavioral sensitization vary across ontogeny. Specifically, nonassociative neuronal processes appear to be

functionally mature in young animals; however, the associative properties modulating the strength of the sensitized response may remain immature during the preweanling period (McDougall et al., 2009a). Two possible explanations for these results exist, either (1) environment-drug (CS-US) pairings are processed differently in preweanling and adult rats (i.e., young rats rely on unitization) or (2) associative properties do not modulate one-trial behavioral sensitization during the preweanling period (McDougall et al., 2011b).

In adult rats, various psychostimulants (e.g., cocaine, amphetamine, methamphetamine, and methylphenidate) are capable of supporting multi- and one-trial behavioral sensitization (Borowsky & Kuhn, 1991; Hirabayashi & Alam, 1981; Kolta et al., 1985; McDougall et al., 1999; Robinson et al., 1982; Weiss et al., 1989). Likewise, preweanling rats are capable of showing multi-trial behavioral sensitization with various psychostimulants (e.g., cocaine, amphetamine, methamphetamine and methylphenidate). Unlike adult rats, however, preweanling rats only exhibit one-trial behavioral sensitization when cocaine is used (McDougall et al., 2011a). Specifically, when preweanling rats were pretreated with cocaine, methamphetamine, methylphenidate,

or amphetamine on PD 19, only cocaine was able to produce one-trial behavioral sensitization on PD 21 (McDougall et al., 2011a). Thus, (1) cocaine-induced behavioral sensitization may be mediated by a specific set of neural mechanisms that are capable of becoming sensitized after a single drug administration, or (2) environment-drug (CS-US) associations may be uniquely supported by cocaine.

Regardless of explanation, it is very surprising that only cocaine, but not methamphetamine, methylphenidate, or amphetamine, was able to induce one-trial behavioral sensitization on PD 21. It is unclear whether this drug-specific effect is unique to this developmental period (PD 19-PD 21) or is a more general characteristic of early ontogeny. Of all the psychostimulants tested in the McDougall et al. (2011a) study, pharmacokinetic properties of cocaine and methamphetamine are the most similar (Brien et al., 1978; Lau, Imam, Ma, & Falk, 1991; Pan & Hedaya, 1998). For this reason, I compared the effects of cocaine and methamphetamine in this thesis. Therefore, one purpose of this thesis was to assess cocaine- and methamphetamine-induced behavioral sensitization during the early (PD 12-13), middle (PD 16-17), and late (PD 20-21) preweanling periods, as well as during preadolescence (PD 24-25), adolescence

(PD 34-35), and adulthood (PD 79-80). The second purpose of this thesis was to determine whether both context-specific and context-independent sensitization would be apparent in the various age groups.

Predictions for this thesis were twofold: First, cocaine-induced, but not methamphetamine-induced, behavioral sensitization would be observed in the early, middle, and late preweanling periods, as well as, in the preadolescent period. In contrast, I predicted that adolescent rats would not exhibit behavioral sensitization and only adult rats would show methamphetamine-induced behavioral sensitization (i.e., methamphetamine would not induce behavioral sensitization in the younger age groups). The bases for these predictions were that preweanling rats show robust cocaine-induced, but not methamphetamine-induced, one-trial behavioral sensitization on PD 20 (McDougall et al., 2011a); whereas, adult rats show strong multi-trial methamphetamine-induced behavioral sensitization (Hirabayashi & Alam, 1981; Kuribara & Uchihashi, 1992). Second, it was hypothesized that cocaine would support context-specific and context-independent behavioral sensitization across the preweanling period, but only context-dependent behavioral sensitization would be exhibited during preadolescence.

Methamphetamine-treated adult rats were predicted to show context-specific, but not context-independent, behavioral sensitization.

CHAPTER EIGHT

MATERIALS AND METHODS

Subjects

Subjects were 336 male and female rats of Sprague-Dawley descent (Charles River, Hollister, CA) that were raised at California State University, San Bernardino (CSUSB). Litters were culled to ten pups on PD 4. Except during testing, rat pups were kept with the dam and littermates in large polycarbonate maternity cages (56 x 34 x 22 cm) with wire lids and Tek-Fresh® bedding (Harlan, Indianapolis, IN). Rats tested at PD 34-35 were weaned at PD 25 and kept with same-sex littermates, while adult rats (i.e., PD 79-80) were also grouped according to sex. Food and water was freely available. The colony room was maintained at 22-24°C and kept under a 12 L:12 D cycle. 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 "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

Behavioral testing was done in commercially available (Coulbourn Instruments, Allentown, PA) activity monitoring chambers (preweanling and preadolescent rats, 25.5 × 25.5 × 41 cm; adolescent and adult rats, 41 × 41 × 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 is 0.76 cm, and the position of each rat was determined every 100 ms.

Drugs

(-)-Cocaine hydrochloride and (+)-methamphetamine hydrochloride were purchased from Sigma (St. Louis, MO). All drugs were dissolved in saline and injected intraperitoneally (IP) at a volume of 5 ml/kg for preweanling and preadolescent rats, while a volume of 1 ml/kg was used for adolescent and adult rats.

Procedure

Experiment 1

Five different age groups were tested: PD 12-13, PD 16-17, and PD 20-21 (early, middle and late preweanling periods, respectively), as well as PD 24-25

(predolescence), and PD 34-35 (adolescence). A total of 24 rats were tested at each of the younger ages, whereas 48 rats were tested on PD 34-35. In order to have sufficient statistical power to detect potential sex differences in sensitized responding, eight male and eight female subjects were assigned to each group on PD 34-35. Prepubescent rats do not typically show drug-induced sex differences (Bowman, Blatt, & Kuhn, 1997; McDougall et al., 2007; Snyder et al., 1998), thus a combined total of eight male and female rats were tested at the younger ages.

During the pretreatment phase, rats from each age group were randomly assigned to one of three training conditions. Rats in the Cocaine-Test groups were taken to the testing room and injected with cocaine (30 mg/kg, IP) immediately before being placed in the activity chambers for 30 minutes. These rats were then returned to the home cage and injected with saline 30 minutes later. Rats in the Cocaine-Home groups were injected with saline before being placed in the activity chambers and injected with cocaine (30 mg/kg, IP) 30 minutes after being returned to the home cage. The Saline Control group received saline in both the activity chamber and home cage. On the pretreatment day, distance traveled was measured for

30 minutes. In all cases, "home" refers to the normal maternity cage that includes both the dam and the littermates.

On PD 13, PD 17, PD 21, PD 25, and PD 35 (i.e., 24 hours after drug pretreatment), all rats (N = 144) received a challenge injection of 20 mg/kg cocaine to determine the occurrence of behavioral sensitization. After cocaine challenge, rats were immediately placed in activity chambers where distance traveled was recorded for 180 minutes.

Experiment 2

For the methamphetamine experiment (N = 192), procedures were similar to those described for Experiment 1, except rats were injected with 4 mg/kg methamphetamine on the pretreatment day and 2 mg/kg on the test day. Aside from the addition of an adult group tested on PD 79-80 (N = 24 males and N = 24 females), the same age groups were used in the two experiments.

Design and Statistical Analysis

For both experiments, pretreatment data for preweanling and preadolescent rats were analyzed using a 2 × 6 (drug × 5-min time block) mixed ANOVA with repeated measures. Test day data was analyzed using a 3 × 18

(condition × 10-min time block) mixed ANOVA with repeated measures. Pretreatment data from the adolescent and adult age groups were analyzed using a 2 × 2 × 6 (drug × sex × 5-min time block) mixed ANOVA with repeated measures; whereas, test day data was analyzed using a 3 × 2 × 18 (condition × sex × 10-min time block) mixed ANOVA with repeated measures. Post hoc analysis of distance traveled data was done using Tukey tests ($P < 0.05$). In all cases, the dependent variable was distance traveled scores.

With all repeated measures ANOVAs, the Huynh-Feldt epsilon statistic was used to adjust degrees of freedom if the assumption of sphericity was violated, as determined by Mauchly's test of sphericity. Corrected degrees of freedom were rounded to the nearest whole number and were indicated by a superscripted "a".

Litter effects were controlled through experimental design. Typically, only one subject from a particular litter was assigned to a given group. In circumstances where more than one subject per litter was found in a particular group (i.e., pretreatment day data) then a single litter mean was calculated from multiple littermates assigned to the same group (Holson & Pearce, 1992; Zorrilla, 1997).

CHAPTER NINE

RESULTS OF EXPERIMENT 1

PD 12-13

Pretreatment Day

Preweanling rats given cocaine (30 mg/kg) exhibited greater distance traveled scores than rats in the saline control group (Figure 1a) [Drug main effect, $F(1, 14) = 13.54$, $P < 0.01$]. This effect varied according to time block, because cocaine-treated rats, when compared to saline-treated rats, only showed an enhanced locomotor response on time block 1 [[a]Drug × Time interaction, $F(3, 40) = 29.65$, $P < 0.001$].

Test Day

Context-specific sensitization was evident on PD 13 because rats in the Cocaine-Test group displayed significantly greater distance traveled scores than rats in the Cocaine-Home and Saline-Control groups (Figure 1b) [Group main effect, $F(2, 21) = 9.38$, $P < 0.01$]. Distance traveled scores varied according to time block, with locomotor activity progressively declining until time block 4 and then increasing at time block 15 [Time main effect, $F(4, 93) = 22.35$, $P < 0.001$].

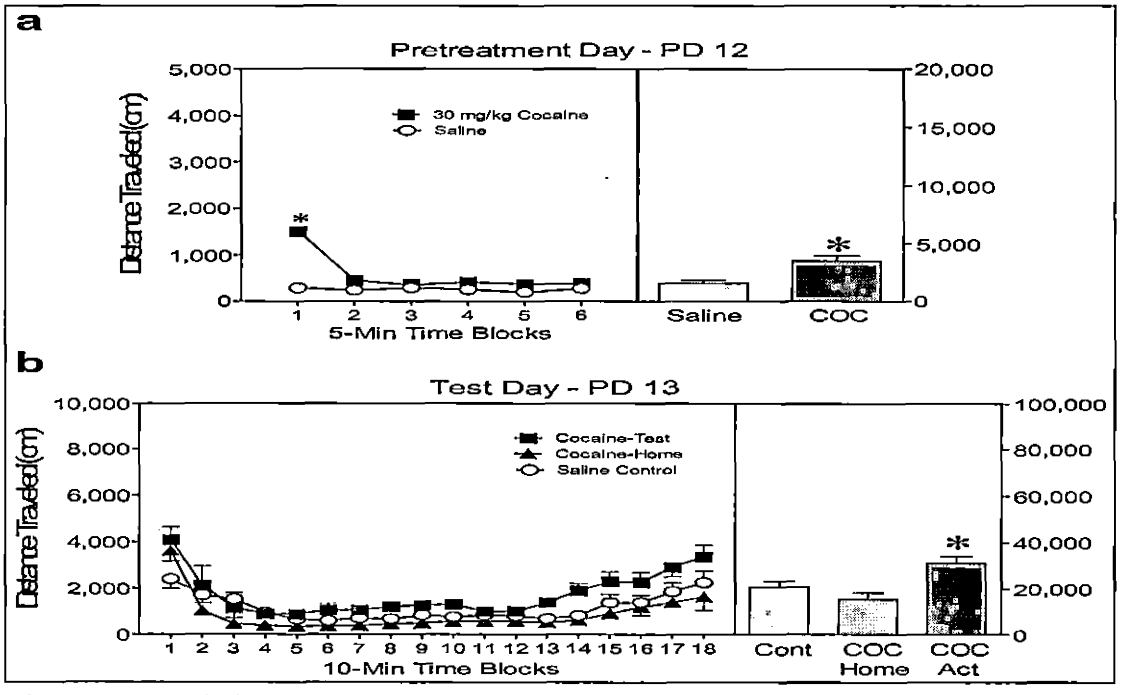

Figure 1. (a) Mean distance traveled scores (±SEM) of preweanling rats injected with saline or 30 mg/kg cocaine before a 30-min placement in the activity chambers on the pretreatment day (i.e., PD 12). (b) Mean distance traveled scores of preweanling rats given a challenge injection of cocaine (20 mg/kg) before the 180-min testing session on PD 13. Rats in the Cocaine-Test group (filled squares) had been pretreated with cocaine (30 mg/kg) before being placed in the activity chamber on PD 12, while rats in the Cocaine-Home group (filled triangles) had been injected with cocaine 30 min after being returned to the home cage. The Saline-Control group (open circles) was injected with saline at both time points. Right panels show mean distance traveled collapsed across the testing sessions. *Significantly different from the control group ($P < 0.05$)

PD 16-17

Pretreatment Day

Rats injected with 30 mg/kg cocaine had significantly greater distance traveled scores than the saline controls (Figure 2a) [Drug main effect, $F(1, 14) = 24.30$, $P < 0.001$]. This effect did not vary according to time block.

Test Day

Behavioral sensitization was evident on PD 17 because rats in the Cocaine-Home and Cocaine-Test groups had significantly greater distance traveled scores than rats given an acute injection of cocaine on the test day (Figure 2b) [Group main effect, $F(2, 21) = 6.10$, $P < 0.01$]. More specifically, the distance traveled scores of the Cocaine-Test and Cocaine-Home groups were significantly greater than the Saline-Control group on time block 1 and time blocks 12-18 [^{a}Drug × Time interaction, $F(10, 109) = 9.21$, $P < 0.001$ and Tukey tests]. On time blocks 2-4, the Saline-Control group had significantly elevated distance traveled scores [Tukey tests].

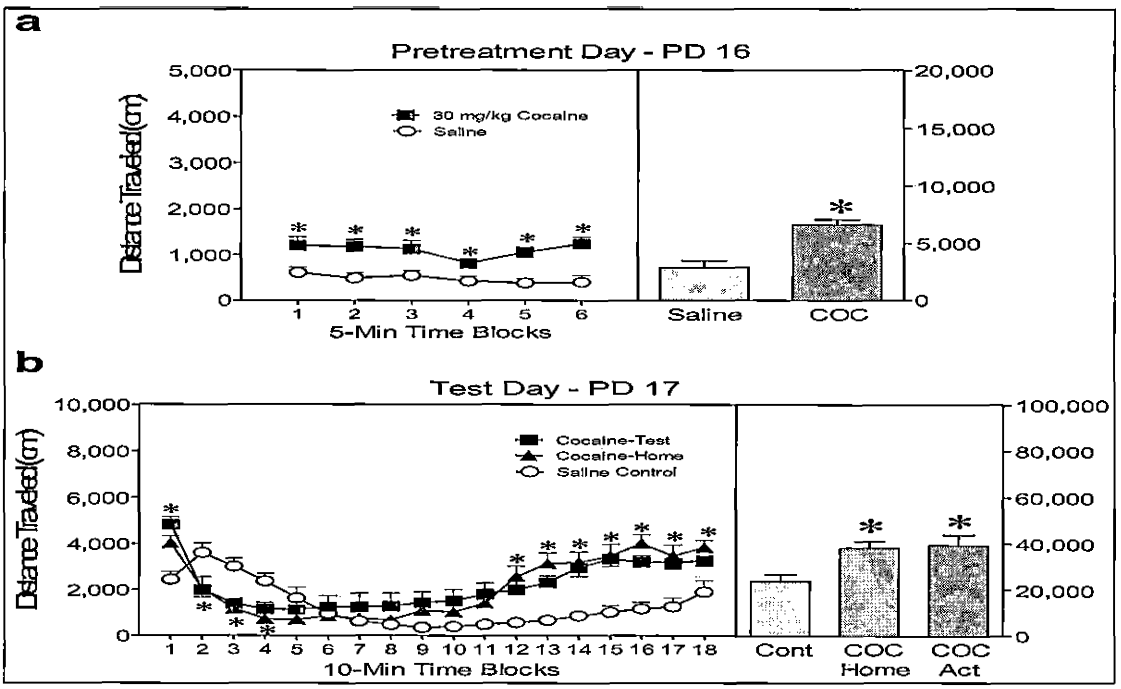

Figure 2. (a) Mean distance traveled scores (±SEM) of preweanling rats injected with saline or 30 mg/kg cocaine before a 30-min placement in the activity chambers on the pretreatment day (i.e., PD 16). (b) Mean distance traveled scores of preweanling rats given a challenge injection of cocaine (20 mg/kg) before the 180-min testing session on PD 17. Rats in the Cocaine-Test group (filled squares) had been pretreated with cocaine (30 mg/kg) before being placed in the activity chamber on PD 16, while rats in the Cocaine-Home group (filled triangles) had been injected with cocaine 30 min after being returned to the home cage. The Saline-Control group (open circles) was injected with saline at both time points. *Right panels* show mean distance traveled collapsed across the testing sessions. *Significantly different from the control group ($P < 0.05$)

Pretreatment Day

Distance traveled scores of preweanling rats injected with 30 mg/kg cocaine were significantly greater than the Saline-Control group (Figure 3a) [Drug main effect, $F(1, 14) = 108.97$, $P < 0.001$]. This effect varied according to time block, with distance traveled scores progressively declining and then stabilizing at time block 3 [Time main effect, $F(3, 44) = 15.44$, $P < 0.001$].

Test Day

Locomotor sensitization was evident on PD 21, because preweanling rats in the Cocaine-Home and Cocaine-Test groups had significantly greater distance traveled scores than the Saline-Control group on the test day (Figure 3b) [Group main effect, $F(2, 21) = 15.28$, $P < 0.001$]. There was a significant difference between the Cocaine-Test and Saline-Control groups, as well as the Cocaine-Home and Saline-Control groups, on time blocks 2-10 [^{a}Drug × Time interaction, $F(5, 55) = 4.05$, $P < 0.01$]. Overall, distance traveled scores declined across the testing session until they stabilized on time block 15 [^{a}Time main effect, $F(3, 55) = 36.29$, $P < 0.001$].

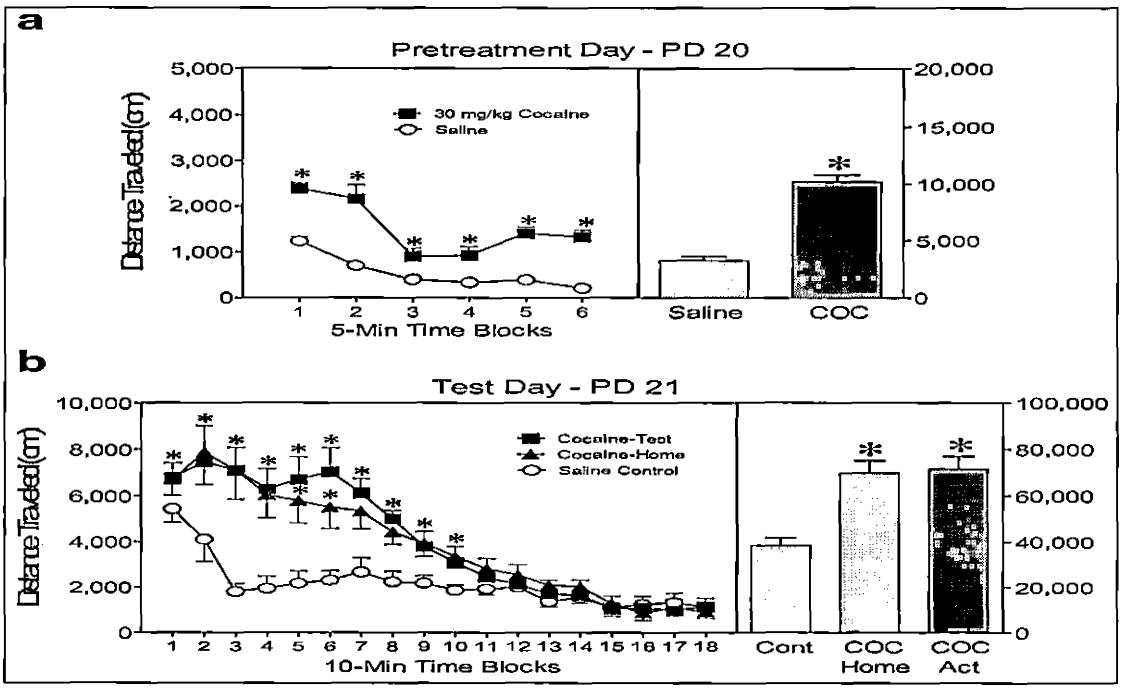

Figure 3. (a) Mean distance traveled scores (±SEM) of preweanling rats injected with saline or 30 mg/kg cocaine before a 30-min placement in the activity chambers on the pretreatment day (i.e., PD 20). (b) Mean distance traveled scores of preweanling rats given a challenge injection of cocaine (20 mg/kg) before the 180-min testing session on PD 21. Rats in the Cocaine-Test group (filled squares) had been pretreated with cocaine (30 mg/kg) before being placed in the activity chamber on PD 20, while rats in the Cocaine-Home group (filled triangles) had been injected with cocaine 30 min after being returned to the home cage. The Saline-Control group (open circles) was injected with saline at both time points. *Right panels* show mean distance traveled collapsed across the testing sessions. *Significantly different from the control group ($P < 0.05$)

PD 24-25

Pretreatment Day

Preadolescent rats given cocaine (30 mg/kg) had significantly greater distance traveled scores than rats given saline (Figure 4a) [Drug main effect, $F(1, 14) = 31.40$, $P < 0.001$]. This effect varied according to time block, because cocaine-treated rats exhibited greater locomotor activity than saline-treated rats on time blocks 1-5 [[a]Drug × Time interaction, $F(3, 43) = 8.52$, $P < 0.001$].

Test Day

On the test day (PD 25), sensitized responding was not evident because rats pretreated and tested with cocaine did not exhibit greater locomotor activity than saline-pretreated rats acutely challenged with 20 mg/kg cocaine (Figure 4b) [Group main effect, $P > 0.05$]. Distance traveled scores varied according to time block, with locomotor activity progressively declining across the testing period [Time main effect, $F(3, 73) = 109.30$, $P < 0.001$].

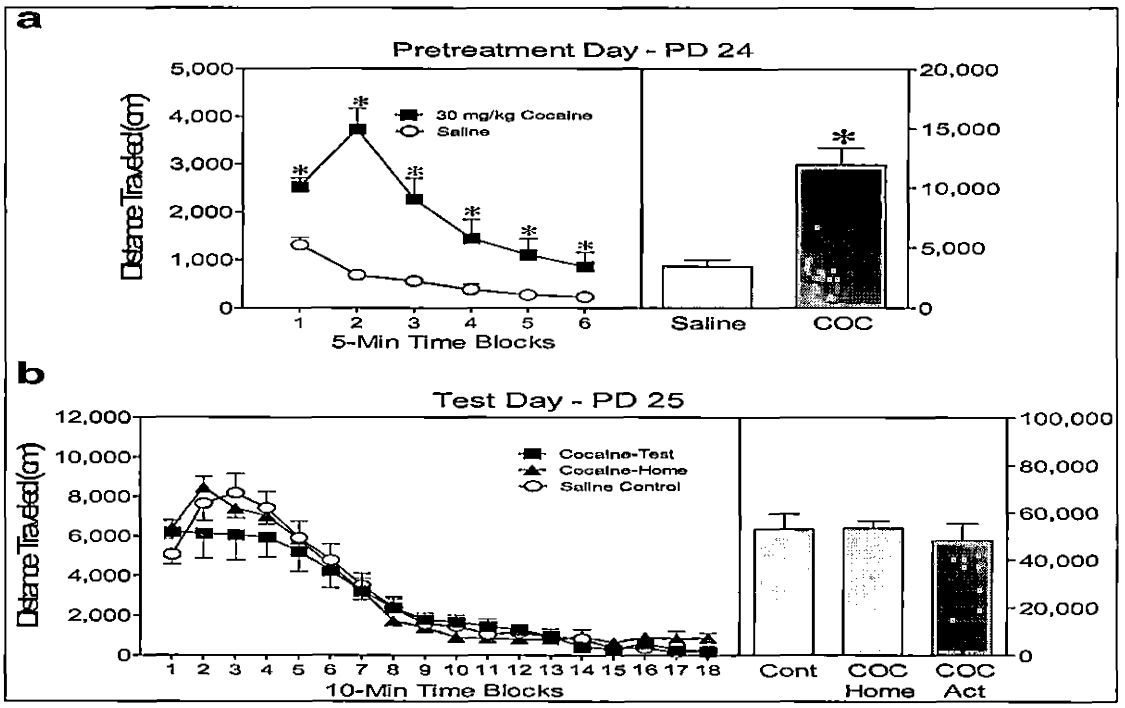

Figure 4. (a) Mean distance traveled scores (±SEM) of preadolescent rats injected with saline or 30 mg/kg cocaine before a 30-min placement in the activity chambers on the pretreatment day (i.e., PD 24). (b) Mean distance traveled scores of preadolescent rats given a challenge injection of cocaine (20 mg/kg) before the 180-min testing session on PD 25. Rats in the Cocaine-Test group (filled squares) had been pretreated with cocaine (30 mg/kg) before being placed in the activity chamber on PD 24, while rats in the Cocaine-Home group (filled triangles) had been injected with cocaine 30 min after being returned to the home cage. The Saline-Control group (open circles) was injected with saline at both time points. *Right panels* show mean distance traveled collapsed across the testing sessions. *Significantly different from the control group ($P < 0.05$)

Pretreatment Day

On PD 34, adolescent rats given 30 mg/kg cocaine had greater distance traveled scores than saline-treated rats (Figure 5a) [Drug main effect, $F(1, 28) = 142.04$, $P < 0.001$]. Cocaine significantly enhanced the locomotor activity of adolescent rats on all six time blocks [[a]Drug x Time interaction, $F(4, 99) = 21.76$, $P < 0.001$]. Neither the main effect nor interactions involving the sex variable were statistically significant.

Test Day

Adolescent rats did not show behavioral sensitization when challenged with 20 mg/kg cocaine (Figure 5b) [Group main effect, $P > 0.05$], with the exception that the Cocaine-Test group exhibited significantly more locomotor activity than the Saline-Control group on time blocks 1 and 15 [[a]Drug x Time interaction, $F(7, 150) = 2.27$, $P < 0.05$]. Overall, rats showed a progressive decline in locomotor activity across the testing period [[a]Time main effect, $F(4, 150) = 190.70$, $P < 0.001$]. Once again, locomotor activity did not vary according to sex.

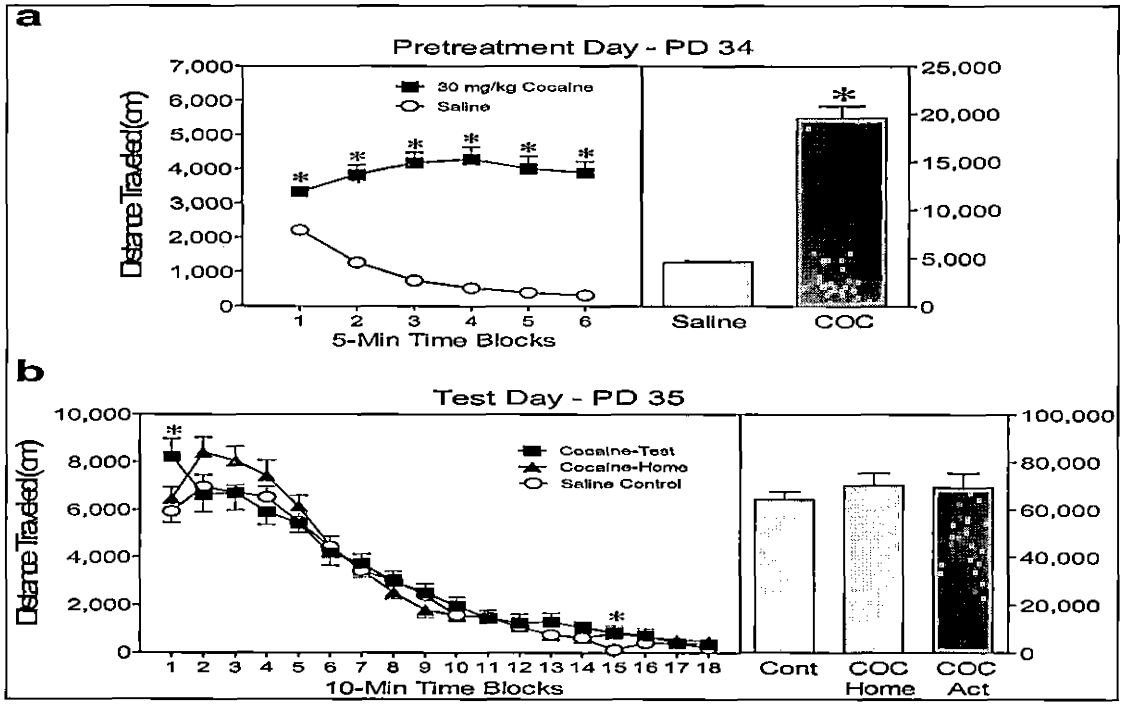

Figure 5. (a) Mean distance traveled scores (±SEM) of adolescent rats injected with saline or 30 mg/kg cocaine before a 30-min placement in the activity chambers on the pretreatment day (i.e., PD 34). (b) Mean distance traveled scores of adolescent rats given a challenge injection of cocaine (20 mg/kg) before the 180-min testing session on PD 35. Rats in the Cocaine-Test group (filled squares) had been pretreated with cocaine (30 mg/kg) before being placed in the activity chamber on PD 34, while rats in the Cocaine-Home group (filled triangles) had been injected with cocaine 30 min after being returned to the home cage. The Saline-Control group (open circles) was injected with saline at both time points. *Right panels* show mean distance traveled collapsed across the testing sessions. *Significantly different from the control group ($P < 0.05$)

CHAPTER TEN

RESULTS OF EXPERIMENT 2

PD 12-13

Pretreatment Day

Rats injected with 4 mg/kg methamphetamine showed significantly greater locomotor activity than preweanling rats in the saline group (Figure 6a) [Drug main effect, $F(1, 14) = 30.88$, $P < 0.001$]. Distance traveled scores varied according to time block, with methamphetamine-treated rats exhibiting greater locomotor activity than saline-treated rats on all 6 time blocks [^{a}Drug × Time interaction, $F(2, 33) = 10.92$, $P < 0.001$].

Test Day

Behavioral sensitization was evident on PD 13 because preweanling rats in the Methamphetamine-Home and Methamphetamine-Test groups had significantly greater distance traveled scores than the Saline-Control group on the test day (Figure 6b) [Group main effect, $F(2, 21) = 16.27$, $P < 0.001$]. There were significant differences between the Methamphetamine-Test and Saline-Control groups, as well as between the Methamphetamine-Home and Saline-Control groups, on time blocks 2-16 [^{a}Drug × Time interaction, $F(12, 126) = 2.21$, $P < 0.05$].

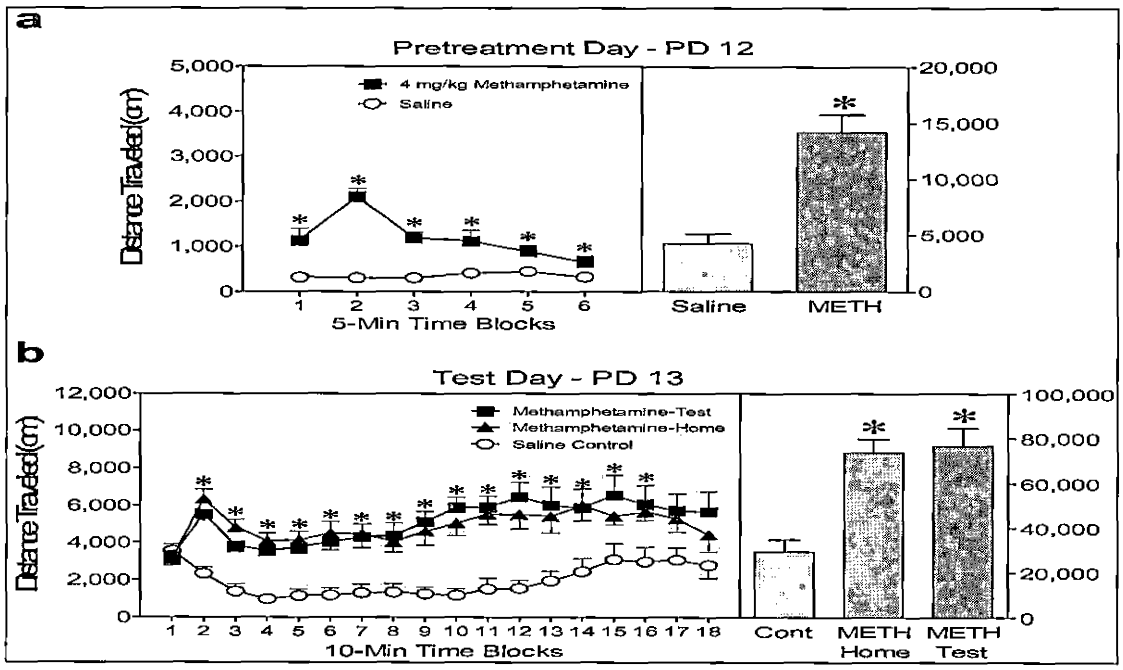

Figure 6. (a) Mean distance traveled scores (±SEM) of preweanling rats injected with saline or 4 mg/kg methamphetamine before a 30-min placement in the activity chambers on the pretreatment day (i.e., PD 12). (b) Mean distance traveled scores of preweanling rats given a challenge injection of methamphetamine (2 mg/kg) before the 180-min testing session on PD 13. Rats in the Methamphetamine-Test group (filled squares) had been pretreated with methamphetamine (4 mg/kg) before being placed in the activity chamber on PD 12, while rats in the Methamphetamine-Home group (filled triangles) had been injected with methamphetamine 30 min after being returned to the home cage. The Saline-Control group (open circles) was injected with saline at both time points. *Right panels* show mean distance traveled collapsed across the testing sessions. *Significantly different from the control group ($P < 0.05$)

PD 16-17

Pretreatment Day

Preweanling rats given 4 mg/kg methamphetamine had significantly greater distance traveled scores than rats in the saline group (Figure 7a) [Drug main effect, $F(1, 14) = 8.81$, $P < 0.05$]. Locomotor activity did not vary according to time block.

Test Day

On the test day (PD 17), locomotor sensitization was evident because rats in the Methamphetamine-Test and Methamphetamine-Home groups had significantly greater distance traveled scores than the Saline-Control group (Figure 7b) [Group main effect, $F(2, 21) = 19.34$, $P < 0.001$]. Preweanling rats in the Methamphetamine-Test and Methamphetamine-Home groups had greater distance traveled scores than rats acutely challenged with methamphetamine (2 mg/kg) on time blocks 2-18 [^{a}Drug $\times$ Time interaction, $F(10, 103) = 2.87$, $P < 0.01$ and Tukey tests].

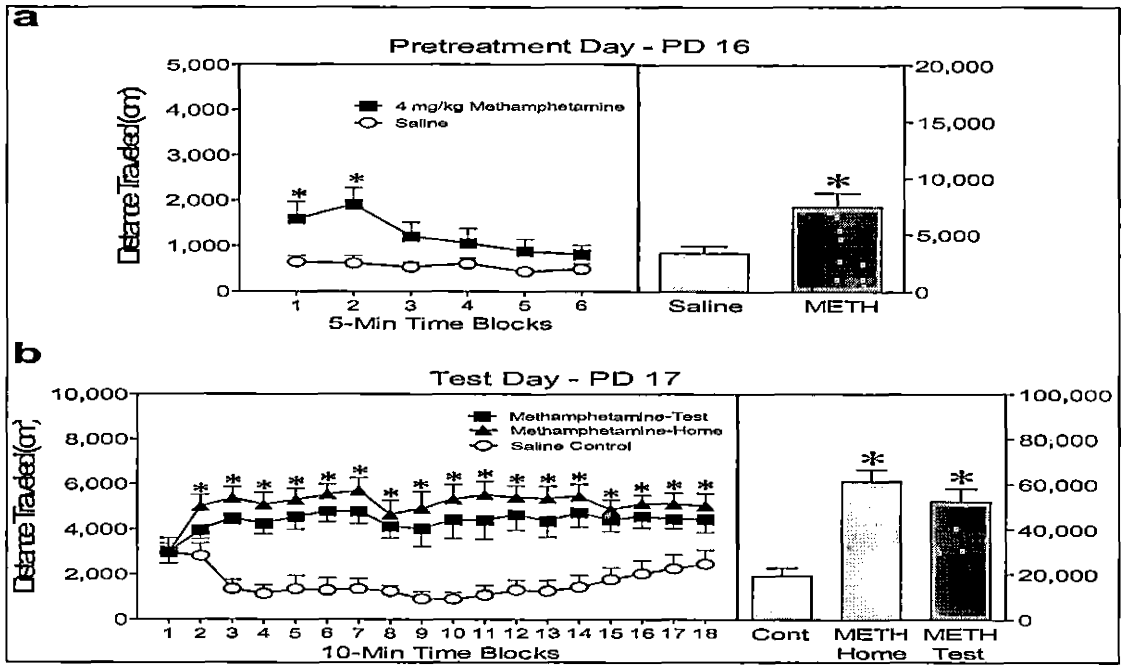

Figure 7. (a) Mean distance traveled scores (±SEM) of preweanling rats injected with saline or 4 mg/kg methamphetamine before a 30-min placement in the activity chambers on the pretreatment day (i.e., PD 16). (b) Mean distance traveled scores of preweanling rats given a challenge injection of methamphetamine (2 mg/kg) before the 180-min testing session on PD 17. Rats in the Methamphetamine-Test group (filled squares) had been pretreated with methamphetamine (4 mg/kg) before being placed in the activity chamber on PD 16, while rats in the Methamphetamine-Home group (filled triangles) had been injected with methamphetamine 30 min after being returned to the home cage. The Saline-Control group (open circles) was injected with saline at both time points. *Right panels* show mean distance traveled collapsed across the testing sessions. *Significantly different from the control group ($P < 0.05$)

PD 20-21

Pretreatment Day

On the pretreatment day (PD 20), methamphetamine-treated rats had significantly greater distance traveled scores than rats in the saline group (Figure 8a) [Drug main effect, $F(1, 14) = 26.46$, $P < 0.001$]. Overall, rats exhibited an initial increase in locomotor activity across the first three time blocks, followed by a decline [^{a}Time main effect, $F(3, 40) = 3.55$, $P < 0.05$].

Test Day

Locomotor sensitization was not evident on PD 21, because preweanling rats in the Methamphetamine-Home and Methamphetamine-Test groups did not have significantly greater distance traveled scores than the Saline-Control group (Figure 8b) [Group main effect, $P > 0.05$]. Locomotor activity varied according to time block, with distance traveled scores increasing across the first three time blocks and then showing a progressive decline [^{a}Time main effect, $F(6, 128) = 30.12$, $P < 0.001$].

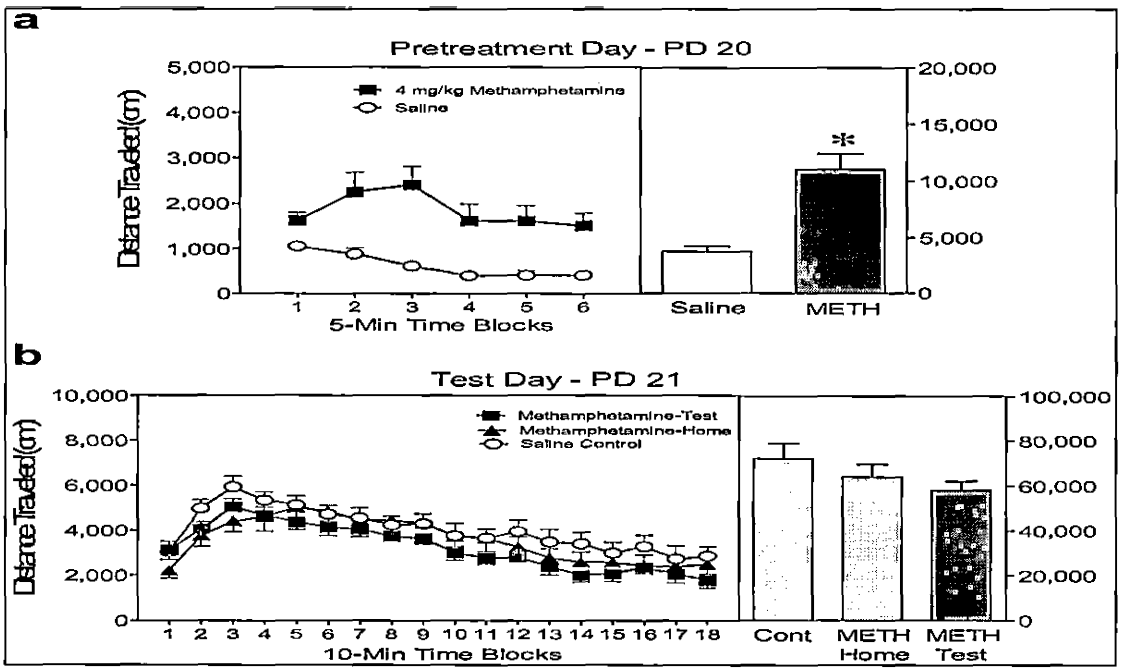

Figure 8. (a) Mean distance traveled scores (±SEM) of preweanling rats injected with saline or 4 mg/kg methamphetamine before a 30-min placement in the activity chambers on the pretreatment day (i.e., PD 20). (b) Mean distance traveled scores of preweanling rats given a challenge injection of methamphetamine (2 mg/kg) before the 180-min testing session on PD 21. Rats in the Methamphetamine-Test group (filled squares) had been pretreated with methamphetamine (4 mg/kg) before being placed in the activity chamber on PD 20, while rats in the Methamphetamine-Home group (filled triangles) had been injected with methamphetamine 30 min after being returned to the home cage. The Saline-Control group (open circles) was injected with saline at both time points. *Right panels* show mean distance traveled collapsed across the testing sessions. *Significantly different from the control group ($P < 0.05$)

PD 24-25

Pretreatment Day

Preadolescent rats injected with 4 mg/kg methamphetamine on the pretreatment day had significantly greater distance traveled scores than rats in the saline group (Figure 9a) [Drug main effect, $F(1, 14) = 90.88$, $P < 0.001$]. More specifically, methamphetamine-treated rats exhibited significantly more locomotor activity than rats in the saline group on all time blocks [^{a}Drug × Time interaction, $F(2, 30) = 10.32$, $P < 0.001$].

Test Day

Behavioral sensitization was not evident on the test day (PD 25), because preadolescent rats in the Methamphetamine-Test and Methamphetamine-Home groups did not have significantly greater locomotor activity than rats in the Saline-Control group (Figure 9b) [Group main effect, $P > 0.05$]. Distance traveled scores varied according to time block, with rats in the Methamphetamine-Test group showing greater locomotor activity than rats in the Methamphetamine-Home group on time block 1. Conversely, rats in the Methamphetamine-Home group exhibited significantly greater distance traveled scores than rats in the Methamphetamine-Test group on time blocks 5 and 6 [^{a}Drug × Time interaction, $F(9, 97) = 2.41$, $P < 0.05$ and Tukey tests].

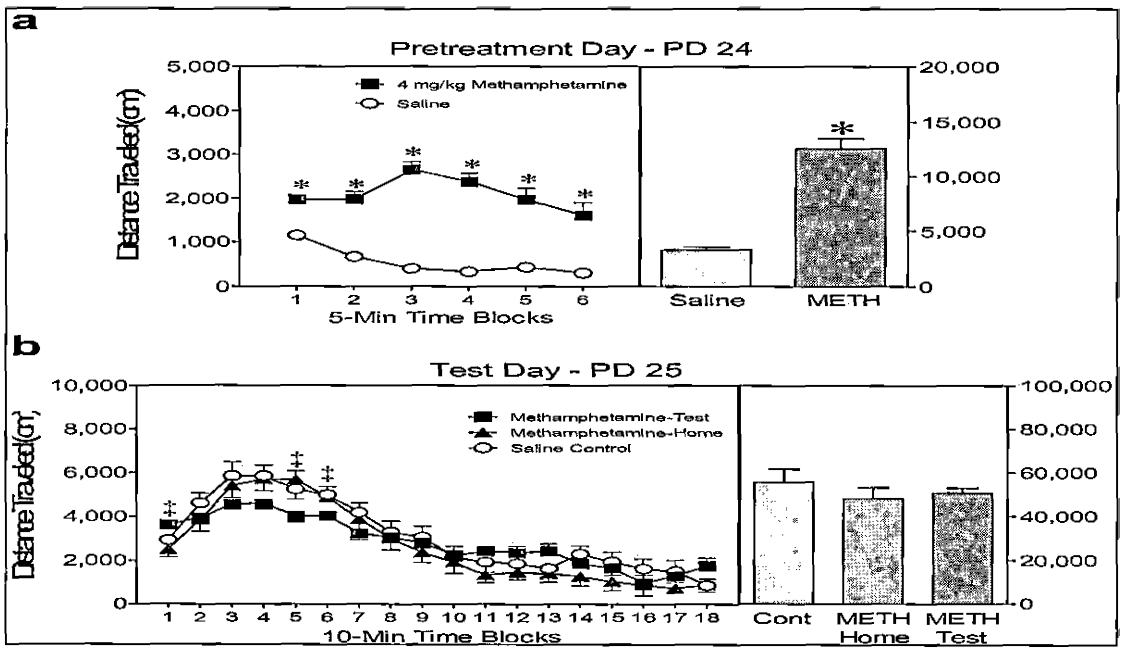

Figure 9. (a) Mean distance traveled scores (±SEM) of preadolescent rats injected with saline or 4 mg/kg methamphetamine before a 30-min placement in the activity chambers on the pretreatment day (i.e., PD 24). (b) Mean distance traveled scores of preadolescent rats given a challenge injection of methamphetamine (2 mg/kg) before the 180-min testing session on PD 25. Rats in the Methamphetamine-Test group (filled squares) had been pretreated with methamphetamine (4 mg/kg) before being placed in the activity chamber on PD 24, while rats in the Methamphetamine-Home group (filled triangles) had been injected with methamphetamine 30 min after being returned to the home cage. The Saline-Control group (open circles) was injected with saline at both time points. *Right panels* show mean distance traveled collapsed across the testing sessions. *Significantly different from the control group ($P < 0.05$). ǂ Significantly different from the Methamphetamine-Test group ($P < 0.05$)

PD 34-35

Pretreatment Day

Rats treated with 4 mg/kg methamphetamine had greater distance traveled scores than rats treated with saline on PD 34 (Figures 10a and 11a) [Drug main effect, $F(1, 28) = 79.12$, $P < 0.001$]. Methamphetamine-treated rats exhibited significantly more locomotor activity than rats in the saline treatment group on time blocks 1-6 [^{a}Drug x Time interaction, $F(3, 75) = 5.19$, $P < 0.01$].

Test Day

Distance traveled scores for PD 35 rats differed according to sex, with females exhibiting greater distance traveled scores than males (Figures 10b and 11b) [sex main effect, $F(1, 42) = 11.46$, $P < 0.01$]. Because locomotor activity differed according to sex, separate analyses for the male and female rats were conducted. Consistent with past literature, methamphetamine-induced behavioral sensitization was not evident in male or female adolescent rats. Methamphetamine-treated male and female rats, however, showed a progressive decrease in locomotor activity across the testing period [Male, ^{a}Time main effect, $F(5, 98) = 33.94$, $P < 0.001$; Female, ^{a}Time main effect, $F(4, 78) = 16.93$, $P < 0.001$].

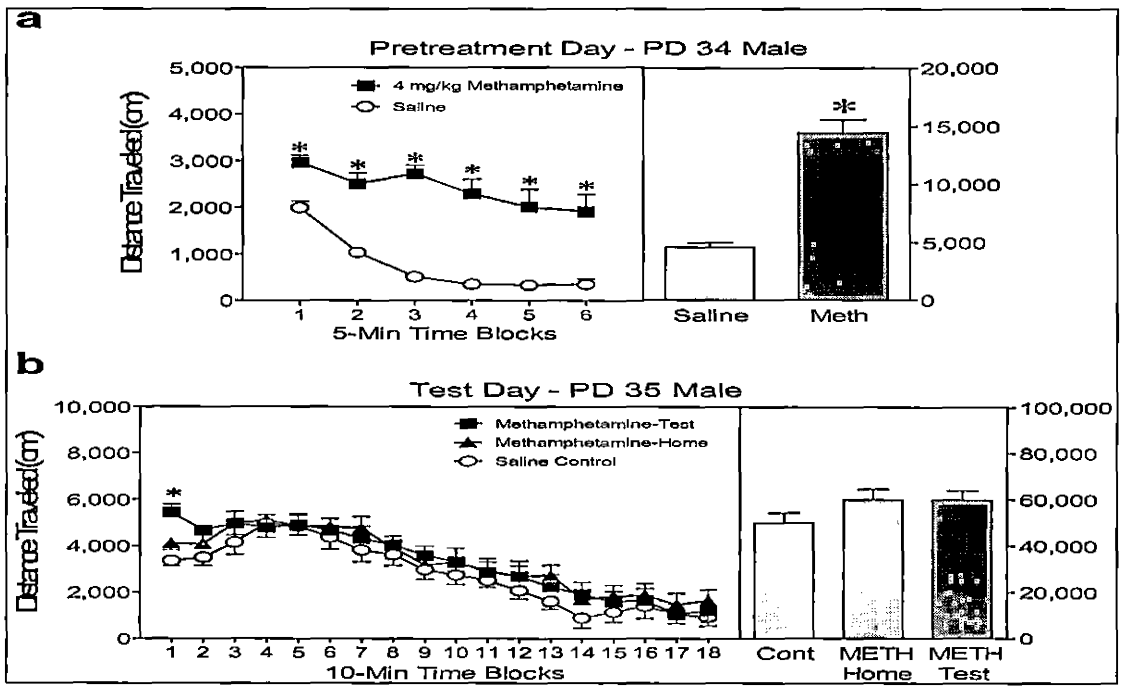

Figure 10. (a) Mean distance traveled scores (±SEM) of male adolescent rats injected with saline or 4 mg/kg methamphetamine before a 30-min placement in the activity chambers on the pretreatment day (i.e., PD 34). (b) Mean distance traveled scores of male adolescent rats given a challenge injection of methamphetamine (2 mg/kg) before the 180-min testing session on PD 35. Rats in the Methamphetamine-Test group (filled squares) had been pretreated with methamphetamine (4 mg/kg) before being placed in the activity chamber on PD 34, while rats in the Methamphetamine-Home group (filled triangles) had been injected with methamphetamine 30 min after being returned to the home cage. The Saline-Control group (open circles) was injected with saline at both time points. *Right panels* show mean distance traveled collapsed across the testing sessions. *Significantly different from the control group ($P < 0.05$)

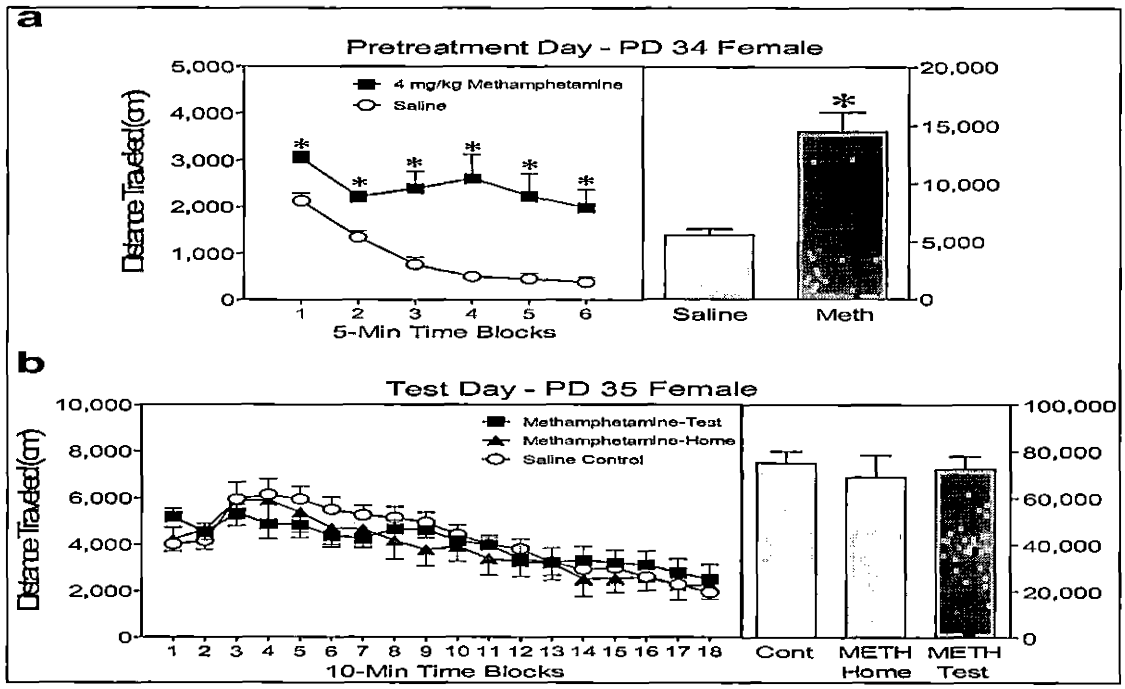

Figure 11. (a) Mean distance traveled scores (±SEM) of female adolescent rats injected with saline or 4 mg/kg methamphetamine before a 30-min placement in the activity chambers on the pretreatment day (i.e., PD 34). (b) Mean distance traveled scores of female adolescent rats given a challenge injection of methamphetamine (2 mg/kg) before the 180-min testing session on PD 35. Rats in the Methamphetamine-Test group (filled squares) had been pretreated with methamphetamine (4 mg/kg) before being placed in the activity chamber on PD 34, while rats in the Methamphetamine-Home group (filled triangles) had been injected with methamphetamine 30 min after being returned to the home cage. The Saline-Control group (open circles) was injected with saline at both time points. *Right panels* show mean distance traveled collapsed across the testing sessions. *Significantly different from the control group ($P < 0.05$)

PD 79-80

Pretreatment Day

On PD 79, distance traveled scores of saline-treated and methamphetamine-treated rats were significantly different (Figures 12a and 13a), with methamphetamine

enhancing locomotor activity [Drug main effect, $F(1, 44) = 51.14$, $P < 0.001$]. In addition, methamphetamine-treated rats had greater distance traveled scores than saline-treated rats on time blocks 2-5 [[a]Drug x Time interaction, $F(3, 123) = 6.68$, $P < 0.001$].

Test Day

On PD 80, behavioral effects differed according to sex, with female rats exhibiting more locomotor activity than male rats (Figures 12b and 13b) [sex main effect, $F(1, 42) = 53.58$, $P < 0.001$]. For this reason, test day data for male and female rats were examined using separate statistical analyses. Female rats in the Methamphetamine-Home and Saline-Control groups exhibited significantly more locomotor activity than rats in the Methamphetamine-Test group on time blocks 4-7 [[a]Drug x Time interaction, $F(7, 73) = 4.80$, $P < 0.001$]; therefore, adult female rats did not exhibit methamphetamine-induced behavioral sensitization. Distance traveled scores for adult males only varied according to time block, with distance traveled scores progressively declining across the testing period [[a]Time main effect, $F(5, 107) = 54.19$, $P < 0.001$].

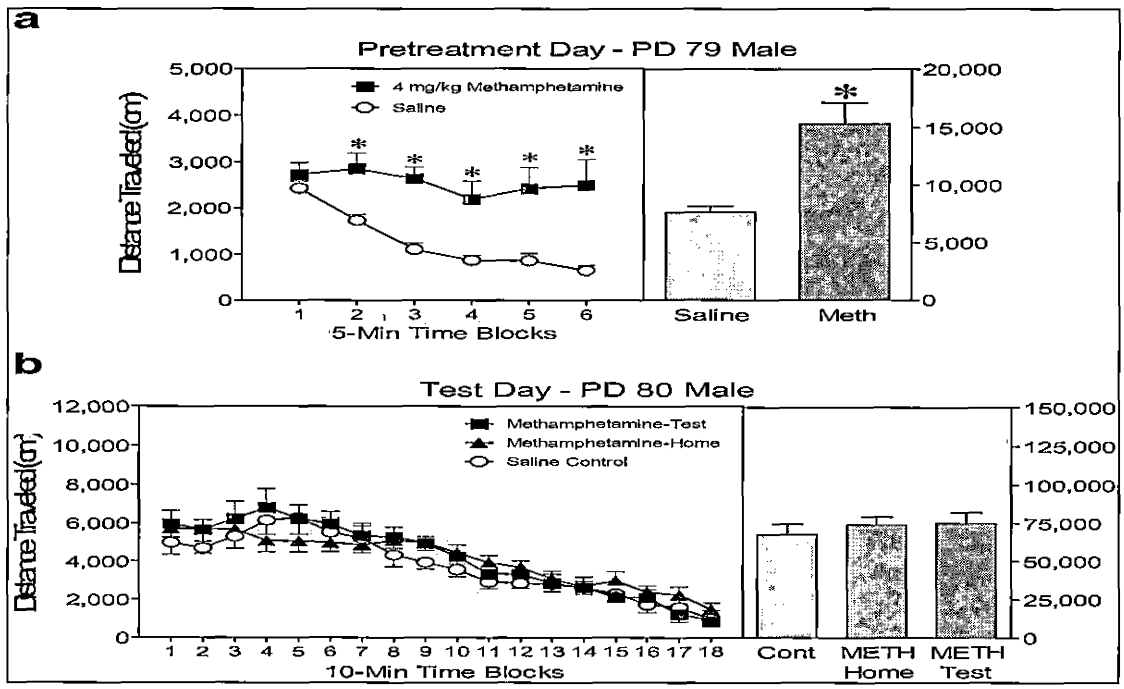

Figure 12. (a) Mean distance traveled scores (±SEM) of male adult rats injected with saline or 4 mg/kg methamphetamine before a 30-min placement in the activity chambers on the pretreatment day (i.e., PD 79). (b) Mean distance traveled scores of male adult rats given a challenge injection of methamphetamine (2 mg/kg) before the 180-min testing session on PD 80. Rats in the Methamphetamine-Test group (filled squares) had been pretreated with methamphetamine (4 mg/kg) before being placed in the activity chamber on PD 79, while rats in the Methamphetamine-Home group (filled triangles) had been injected with methamphetamine 30 min after being returned to the home cage. The Saline-Control group (open circles) was injected with saline at both time points. *Right panels* show mean distance traveled collapsed across the testing sessions. *Significantly different from the control group ($P < 0.05$).

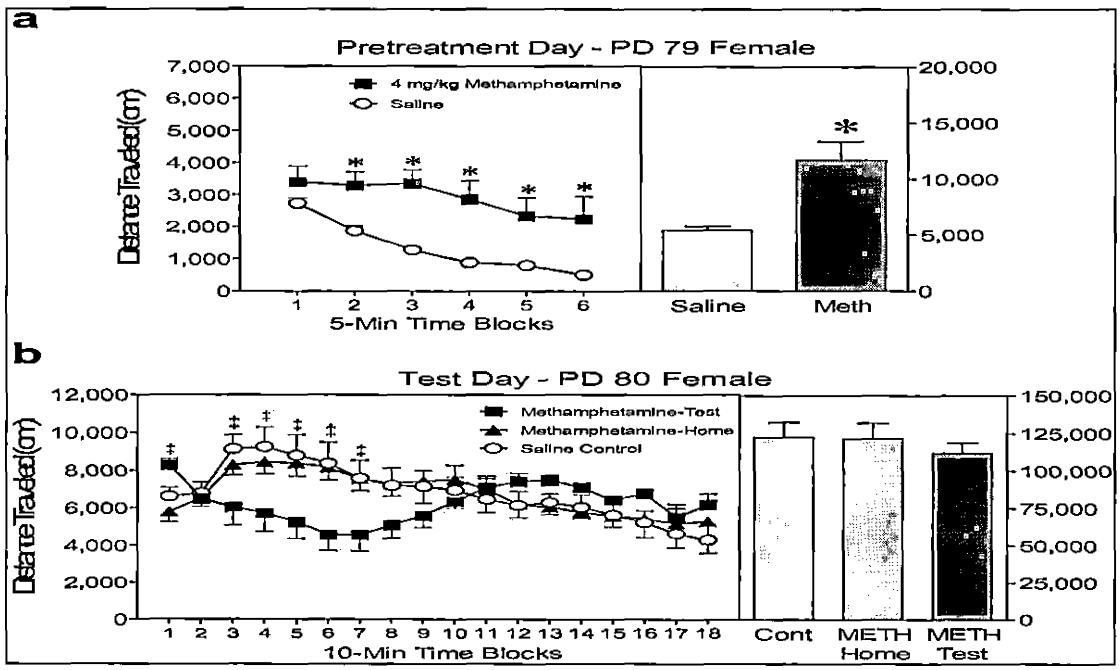

Figure 13. (a) Mean distance traveled scores (±SEM) of female adult rats injected with saline or 4 mg/kg methamphetamine before a 30-min placement in the activity chambers on the pretreatment day (i.e., PD 79). (b) Mean distance traveled scores of preadolescent rats given a challenge injection of methamphetamine (2 mg/kg) before the 180-min testing session on PD 80. Rats in the Methamphetamine-Test group (filled squares) had been pretreated with methamphetamine (4 mg/kg) before being placed in the activity chamber on PD 79, while rats in the Methamphetamine-Home group (filled triangles) had been injected with methamphetamine 30 min after being returned to the home cage. The Saline-Control group (open circles) was injected with saline at both time points. *Right panels* show mean distance traveled collapsed across the testing sessions. *Significantly different from the control group ($P < 0.05$). ‡ Significantly different from the Methamphetamine-Test group ($P < 0.05$)

CHAPTER ELEVEN

DISCUSSION

It was hypothesized that one-trial cocaine-induced behavioral sensitization would be observed in the early, middle, and late preweanling periods. As expected, an augmented locomotor response was evident when cocaine-pretreated rats were given a challenge injection of cocaine on PD 13, PD 17, and PD 21. Preadolescent and adolescent rats, however, did not exhibit one-trial behavioral sensitization when challenged with cocaine on PD 25 or PD 35.

Contrary to predictions, one-trial methamphetamine-induced locomotor sensitization was evident during the early (PD 12-13) and middle (PD 16-17) preweanling periods. Unlike what was observed with cocaine, one-trial behavioral sensitization was not evident when rats were pretreated and then challenged with methamphetamine on PD 21. Consistent with the cocaine data, repeated methamphetamine treatment did not induce behavioral sensitization in preadolescent and adolescent rats. Although one-trial methamphetamine-induced locomotor sensitization was not evident in adult rats, methamphetamine did cause a greater locomotor response in

female rats than males. Of course, caution must be used to interpret these data because only one drug dose was used across all ages.

In general, the methamphetamine- and cocaine-induced sensitized responding of preweanling rats was not affected by environmental conditioning factors. More specifically, rats showed a sensitized response regardless of whether cocaine or methamphetamine pretreatment occurred in the activity chamber or home cage. The one exception involved rats from the early preweanling period, because only context-dependent cocaine-induced behavioral sensitization was evident on PD 13.

Based on results from this thesis and other studies, it can be concluded that the importance of environmental conditioning factors varies across ontogeny. Most obviously, preweanling rats are capable of showing one-trial context-independent behavioral sensitization, whereas adult rats only exhibit context-specific behavioral sensitization. A possible explanation for this ontogenetic difference is that the nonassociative neural adaptations underlying sensitized responding are functionally mature in young animals; whereas, associative processes modulating the strength of the sensitized response remain immature (present study; McDougall et al.,

2009a). The nature of this proposed associative immaturity is uncertain, but it is possible that:
(1) environment-drug (CS-US) pairings are processed differently across ontogeny (i.e., young rats rely on unitization) or (2) one-trial behavioral sensitization is not modulated by associative processes during the preweanling period.

The first explanation relies on the concept of "unitization", in which preweanling rats treat discrete stimuli equivalently if multiple CSs are paired with the same US. In other words, preweanling rats exhibit context-independent behavioral sensitization because they perceive the drug-paired home cage and the test chamber as being equivalent. The unitization explanation, for the most part, is consistent with much of my thesis data; however, this model cannot explain why PD 13 rats showed only context-specific behavioral sensitization when tested with cocaine. The latter effect may result from the dissimilarity between the home cage and the activity chamber. More Specifically, the augmented locomotor response exhibited by PD 13 rats may be a consequence of a stress or fear response caused by initial exposure to the novel environment (Campbell & Raskin, 1978; Zavala et al., 2000). A second possibility is that a single

drug-environment pairing is insufficient to induce associative conditioning in preweanling rats. If true, nonassociative neural mechanisms are exclusively responsible for the development and expression of sensitized responding during the preweanling period. During adulthood a different system is operating, because associative properties are necessary for the one-trial behavioral sensitization of adult rats. Specifically, sensitized responding is not evident when pretreatment and testing occur in distinctly different environments (Battisti et al., 1999; Jackson & Nutt, 1992; McDougall et al., 2007; Weiss et al., 1989).

The present results also show that the induction of one-trial behavioral sensitization in preweanling rats is not unique to a specific psychostimulant. McDougall et al. (2011a) reported that only cocaine, but not methamphetamine, was able to produce one-trial behavioral sensitization during the late preweanling period (i.e., on PD 21). Results from my thesis both confirm this earlier finding and indicate that the lack of methamphetamine-induced behavioral sensitization is unique to a specific developmental period and is not a general characteristic of early ontogeny. More specifically, rats tested during the early and middle preweanling periods

(i.e., from PD 12 to PD 17) showed robust locomotor sensitization when pretreated and challenged with methamphetamine, but rats tested on PD 21, PD 25, and PD 35 did not exhibit a methamphetamine-induced sensitized response. Although both cocaine and methamphetamine supported behavioral sensitization in the present thesis, some important drug-specific effects were apparent. For example, methamphetamine caused robust behavioral sensitization on PD 13 and PD 17, whereas cocaine produced weak sensitized responding on PD 13 and strong behavioral sensitization on PD 17 and PD 21.

Differences in the ontogeny of cocaine- and methamphetamine-induced behavioral sensitization may be due to drug affinity and/or pharmacokinetic factors. Although both cocaine and methamphetamine have an approximately equal affinity for dopamine and norepinephrine transporters, methamphetamine has a relatively lower affinity for the serotonin transporter than cocaine (Howell & Kimmel, 2008). This difference in affinity could impact the pattern of sensitized responding, because serotonergic functioning alters dopamine system activity and, in turn, modulates the behavioral effects of psychostimulants (Pierce & Kalivas, 1997; Robinson & Becker, 1986). Moreover, agonist- and

antagonist-induced alterations in serotonin system functioning facilitate or inhibit the sensitization process (Ago, Nakamura, Hayashi, Itoh, Baba, & Matsuda, 2006; Szumlinksi, Frys, & Kalivas, 2004).

In the rat CNS, the serotonergic system starts to develop by gestational day (G) 11-12, whereas dopaminergic markers appear around G 13-14 (Lauder, 2006; Noisin & Thomas, 1988). Dopamine and serotonin systems interact to promote axon growth and synapse formation across gestation and postnatal development (Herregodts, Velkeniers, Ebinger, Michotte, Vanhaelst, & Hooghe-Peters, 1990; Lauder, 2006; Noisin & Thomas, 1988). Later in development, the serotonin and dopamine systems co-modulate cortical neurons and influence one another at the level of their respective brainstem nuclei (Benes, Taylor, & Cunningham, 2010). In terms of the present study, the different patterns of cocaine- and methamphetamine-induced behavioral sensitization may be explained by the differential maturation of the dopamine and serotonin systems, both alone and as an integrated functional unit. Specifically, dopamine systems underlying methamphetamine-induced sensitization may develop earlier (i.e., allowing expression of methamphetamine-induced sensitization in PD 13 rats), but have a relatively

short-lived activation period (i.e., lasting through only the middle preweanling period). Conversely, cocaine, which activates both dopamine and serotonin systems, may have a developmentally later onset of action (i.e., cocaine produces more robust sensitization in the middle preweanling period), yet have a longer-lasting activation period (i.e., cocaine-induced behavioral sensitization lasts through the late preweanling period).

In contrast to preweanling rats, preadolescent and adolescent rats did not show any evidence of cocaine- or methamphetamine-induced behavioral sensitization after a single drug-environment pairing. Consistent with my results, Collins and Izenwasser (2004) also report the absence of psychostimulant-induced behavioral sensitization across the adolescent period. The lack of sensitized responding during preadolescence and adolescence may be due to age-dependent changes in pharmacokinetics (Spear, 2000). In particular, puberty causes changes in gonadal steroid titers that may, in turn, alter the excretion rate, distribution, and metabolism of psychostimulants (Hein, 1987).

Ontogenetic changes in the neural substrate may also contribute to differences in psychopharmacological responsiveness during the adolescent period. Specifically,

many neural systems affected by environmental conditioning undergo maturational change during adolescence. Examples of these modifiable systems include the mesolimbic and mesocortical pathways, which are critical for regulating the reward value of psychostimulants (Abercrombie, Keefe, DiFrischia, & Zigmond, 1989; Cuadra, Zurita, Lacerra, & Molina, 1999; Dunn & Kramarcy, 1984; Thierry, Tassin, Blanc, & Glowinski, 1976). Evidence that these neural systems undergo modification during adolescence is provided by studies showing that adolescent rats and mice are less sensitive to an acute injection of a psychostimulant (i.e., amphetamine and cocaine) than younger or older animals (Bolanos, Glatt, & Jackson, 1998; Lanier & Isaacson, 1977; Laviola, Adriani, Terranova, & Gerra, 1999; Laviola, Wood, Kuhn, Francis, & Spear, 1995; Spear & Brick, 1979). Interestingly, however, *repeated* treatment with a psychostimulant may induce greater behavioral responsiveness in adolescent rats than adults (Caster, Walker, & Kuhn, 2007).

Methamphetamine-treated adult rats did not show one-trial behavioral sensitization when tested on PD 80. This result contrasts with previous studies showing that adult rats do exhibit one-trial cocaine-induced behavioral sensitization (Fontana et al., 1993; McDougall et al.,

2007, 2009a; Weiss et al., 1989). Several explanations can account for adult rats exhibiting cocaine-induced, but not methamphetamine-induced, one-trial locomotor sensitization. First, cocaine-induced behavioral sensitization may be mediated by neural mechanisms that are capable of becoming sensitized after a single pretreatment trial. In contrast, methamphetamine-induced behavioral sensitization may rely on different neural pathways that cannot be sensitized after a single exposure to the psychostimulant. Second, the pharmacodynamics of cocaine may make this drug uniquely able to support environment-drug (CS-US) associations in adulthood (i.e., one-trial sensitization in adult rats relies on associative conditioning). Finally, methamphetamine, at the doses tested, could have preferentially induced a sensitized stereotyped response rather than a locomotor response.

Although adult rats did not show methamphetamine-induced locomotor sensitization, responsiveness to methamphetamine varied according to sex. Adult female rats exhibited more locomotor activity than male rats, which is consistent with many studies showing that female rats are more sensitive to psychostimulants than males (Peris, Decambre, Coleman-Hardee, & Simpkins,

1991; Sell, Scalzitti, Thomas, & Cunningham, 2000; Ujike, Tsuchida, Akiyama, Fujiwara, & Kuroda, 1995). Ovarian steroid hormones in females going through estrus cause an enhanced sensitivity to cocaine (Peris et al., 1991; Sell et al., 2000). Estradiol increases dopamine release and reuptake in the striatum, alters dopamine neurotransmission and, in turn, modulates psychostimulant-induced behaviors (Becker, 1990; Castner, Xiao, & Becker, 1993; Chiodo, Caggiula, & Saller, 1981; Dluzen & Ramirez, 1990; Hruska, Ludmer, Pitman, Ryck, & Silbergeld, 1982; McDermott, 1993; Thompson & Moss, 1994).

During adulthood, gonadal hormones may be responsible for gender differences observed after psychostimulant treatment (Ujike et al., 1995). For example, administering estradiol to ovariectomized female rats intensifies amphetamine-induced behavioral sensitization, including stereotypy (Camp, Becker, & Robinson, 1986; Chiodo et al., 1981; Savageau & Beatty, 1981). Similarly, the ability of ovarian hormones to modulate the effects of psychostimulants may explain the sex-differences found in my thesis. If we had controlled hormonal cycling, it is possible that our adult female rats would have shown an augmented behavioral response after repeated methamphetamine treatment.

In conclusion, the most notable results coming out of this thesis involve ontogenetic changes in the expression of one-trial cocaine- and methamphetamine-induced behavioral sensitization. Specifically, robust sensitized responding is observed at young ages and completely disappears during the preadolescent and adolescent periods. The ontogenetic shift in the expression of behavioral sensitization could be attributed to: (1) age-dependent changes in pharmacokinetic factors (perhaps due to the influence of sex hormones), or (2) differences in psychopharmacological responsiveness caused by ontogenetic changes in the neural substrate. Regardless of underlying causes, this thesis further confirms that locomotor sensitization is not evident during the preadolescent and adolescent periods (see also Collins & Izenwasser, 2004). Furthermore, this thesis extends the results of previous studies (i.e., McDougall et al., 2011a) by showing that methamphetamine-induced behavioral sensitization is evident during the early and middle preweanling periods, but disappears by the late preweanling period and beyond.

The present thesis is also germane to human drug addiction. Although various ontogenetic stages in the rat have translational relevance to humans, the late

preweanling period is of particular interest because it is approximately analogous to late childhood in humans (Smith & Morrell, 2008). According to a survey conducted in 2005, about 7% of United States children in this developmental stage have illicitly sampled psychostimulants or other drugs of abuse (Johnston, O'Malley, Bachman, & Schulenberg, 2005; Smith & Morrell, 2008). There is growing evidence showing that early illicit drug experimentation (e.g., during late childhood), can eventually progress to regular drug use and lead to a higher likelihood of drug addiction (Grant & Dawson, 1997; Wagner & Anthony, 2002; Warner & White, 2003).

The transition from simple drug use to addiction is much quicker in youth than adults (Smith & Morrell, 2008; Spear, 2000), potentially implying that these ontogenetic differences in drug responsiveness may have significant consequences during adolescence. Specifically, drug consumption during adolescence is often characterized by episodes of bingeing (Spear, 2000), which may be the result of reduced sensitivity to the psychostimulant (Smith & Morrell, 2008; Spear, 2000). In general, results from my thesis highlight the risks involved in early psychostimulant use and show that mechanisms associated

with addiction (i.e., behavioral sensitization) are operating during early ontogeny.

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