EXPOSURE TO METHAMPHETAMINE AND PHENCYCLIDINE DURING DEVELOPMENT AND SUBSEQUENT BEHAVIORAL CHANGE

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Takehiro Minamoto

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Director of Thesis

Master's Committee: _____, Chair

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Date

EXPOSURE TO METHAMPHETAMINE AND PHENCYCLIDINE DURING DEVELOPMENT AND SUBSEQUENT BEHAVIORAL CHANGES

Takehiro Minamoto, M.S.
Morehead State University, 2005

Director of Thesis: _____

Abstract

In adult rats, high doses of methamphetamine (METH) and phencyclidine (PCP) produce neurological damage in the central nervous system and subsequent behavioral deficits. These deficits are thought to be due to changes in the neurotransmitter systems, such as dopamine, serotonin, and glutamate. Studies have suggested that exposure to METH and PCP during early development produces behavioral deficits. However, it is unclear if exposure to these drugs during later development also produces similar behavioral deficits.

The present study examined the effects of brief exposure to METH and PCP during later development and subsequent changes in behavior. Rats on postnatal days 50-51 were exposed to METH and PCP. To measure short- and long-term effects of drugs on behavior three experiments were conducted using different behavioral tests: locomotor activity, social interaction, and spatial learning. Rats were housed together in a gang cage from postnatal day (PD) 30 until PD80. On PD 80, rats were housed individually in single cages shortly prior to the learning experiment, which involved

food-deprivation. Experiment 1: Locomotor activity was measured during the acute drug sate and the withdrawal period. On PD 50-51, rats received METH (9 mg/kg), PCP (9 mg/kg), or saline. A total of four injections were done subcutaneously at a 12 hr interval (twice/day, 2 days). Using a video-tracking system locomotor activity was tested in an open field arena for 60 min at multiple times: acute state (immediately after the first and the third injection) and withdrawal state (3, 7, 14 and 28 days after the last injection). The first METH injection enhanced locomotion during the first half of the session, but not the second half, whereas the third injection of METH did not affect locomotion during the entire session. The first PCP injection did not affect locomotion during the first half of the session, but increased locomotion during the second half, whereas the third injection of PCP further enhanced locomotion during the entire session. Locomotor activity of METH and PCP groups was comparable to that of vehicle group after withdrawal Day 3. Experiment 2: Social interaction was measured during the withdrawal period. The schedule of METH and PCP treatment (age of rats, dose, frequency, interval, and mode) was identical to Experiment 1. Social interaction was measured by the frequency and the duration of the contact during a 60 min period. METH-treated rats showed a gradual decrease in social interaction on Day 7-14 of withdrawal. PCP-treated rats showed a decreasing trend in social interaction during the initial contact, the first 8 min observation period. Experiment 3: Spatial and reversal learning were measured in adulthood, after PD 90. The schedule of METH and PCP treatment (age of rats, dose, frequency, interval, and mode) was identical to Experiment 1 and 2. To test spatial learning, rats were trained

in a spatial discrimination task, which required a barpress opposite to the cue location to receive a food pellet. Once their performance reached a criterion (≥85% correct responses, 3 sessions), rats were trained in the reversal task, which required a barpress same as the cue location. Neither METH nor PCP affected spatial discrimination. During reversal, however, METH-treated rats tended to show a retarded acquisition, whereas PCP significantly impaired reversal learning.

The present study demonstrates that exposure to METH and PCP on PD50-51 affected locomotor activity during the acute drug phase but not during withdrawal. However, METH and PCP during later development decreased social interaction during the withdrawal period, and selectively impaired reversal learning in adulthood.

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Morehead State University

Takehiro Minamoto

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Exposure to Methamphetamine and Phencyclidine during Development and Subsequent Behavioral Change

Methamphetamine (METH) is an amphetamine derivative, which enhances dopamine (DA) transmission in the brain. Phencyclidine (PCP) is a glutamate antagonist, which blocks N-methyl-D-aspartate (NMDA) receptors in the central nervous system (Zukin & Javitt, 1993). Despite their action on different neurotransmitter systems, methamphetamine and PCP appear to produce similar behavioral changes acutely and chronically. Such behavioral changes differ characteristically during acute and chronic states and also differ depending on the doses tested. For example, METH affects spontaneous locomotion (Clemens et al., 2004) and produces neurotoxic effects at high doses by depleting DA and serotonin (5-HT) in several brain regions, including the striatum, the nucleus accumbens, and the prefrontal cortex (Friedman et al., 1998; Gehrke et al., 2003). Repeated METH administration leads to long-term behavioral and neurochemical changes with a decrease in DA and 5-HT and their metabolites in the brain, while chronic PCP results in upregulation of NMDA receptors in limbic regions, such as the prefrontal cortex and the hippocampus (Bisagno et al., 2002; Friedman et al., 1998; Sircar, 2003; Yu et al., 2002). These studies suggest that repeated administration of METH and PCP produce neurological changes in the central nervous system in addition to behavioral changes.

Effects of Methamphetamine and PCP on Locomotor Activity: Acute and Withdrawal Phases

Methamphetamine and Locomotor Activity. In adult rats, an acute injection of methamphetamine (METH) enhances locomotor activity (Clemens et al., 2004; Ohmori et al., 1995). However, METH effects on locomotor activity (hyperlocomotion) vary depending on the dose. At a low dose (0.3-2.0 mg/kg), METH enhances locomotion, whereas a moderate dose of METH (3.0-4.0 mg/kg) increases locomotion and induces stereotypy (Gentry et al., 2004; Shoblock et al., 2003). In developing rats, however, the acute effects of METH have not been tested. However, findings from a previous study showed that the effects of amphetamine, a similar psychostimulant, on locomotor activity vary depending on age. For example, low to high doses of amphetamine (2-10 mg/kg) enhanced locomotor activity on postnatal days (PD) 18-22, but the same doses failed to affect locomotion after PD 34-38 (Lanier & Isaacson, 1977). For older rats (PD 45-49) medium doses (2-5 mg/kg) enhanced locomotion, whereas a high dose (10 mg/kg) did not affect locomotor activity (Lanier & Isaacson, 1977). Thus, given that METH and amphetamine act on the central nervous system in a similar manner (Melega et al., 1995), a prediction is that the acute effects of METH on locomotor activity may also vary across different age groups. Although acute METH produces differential effects on locomotion depending on the dose in adult rats, METH effects on locomotor activity in developing rats are not clear.

During the withdrawal period, locomotor activity of METH-treated rats

appears to be biphasic. Timar et al. (2003) reported that in adult rats, suppressed locomotion was observed during the first 3 days of withdrawal, but not 7, 14, and 28 days after METH administration (10 mg/kg, 4 injections). However, Wallance et al. (2001) found in adult rats a decrease in locomotion during 7-13 days after METH injections using the same dose and frequency used in Timar et al's study (2003). In Wallance et al's study, locomotor activity was measured continuously for 24 hrs, and locomotion was decreased during the diurnal period, but not during the nocturnal period, suggesting that the time of behavioral testing contributes to METH effects on locomotion.

In developing rats, METH effects on locomotor activity during the withdrawal period appear to differ from those for adult rats. METH exposure during PD 1-10 or PD 11-20 (30 mg/kg, two injections/day, 10 days) produced hypolocomotion when tested on PD 60 (Vorhees et al., 1994) which corresponds to 30-50 days of withdrawal. These results suggest that exposure to METH during development produce a prolonged effect on locomotion. Thus, although exposure to high doses of METH appears to suppress locomotion during withdrawal periods in both adult and developing rats, the impact of METH on behavior is greater on developing rats than adult rats.

Neurochemical Change Associated with METH. METH-induced hyperlocomotion is thought due to enhance dopamine (DA) and serotonin (5-HT) in the brain, particularly in the nucleus accumbens (NAc). Microdialysis studies have indicated that METH enhanced locomotion and also increased DA and 5-HT in the NAc

(Shimada et al., 1996; Shoblock et al., 2003). This is consistent with the notion that DA in the NAc mediates locomotor activity (Wise, 2000). Further evidence showed that depletion of DA in the NAc inhibited hyperlocomotion induced by a dopamine agonist, apomorphine (Liu et al., 1998). On the other hand, effects on 5-HT may affect locomotion indirectly via the DA system. For example, direct infusion of 5-HT_{1A} agonist into the NAc potentiated hyperlocomotion induced by cocaine, a dopamine agonist, while 5-HT_{1A} agonist alone did not affect locomotion (Muller et al., 2004). Thus, it appears that 5-HT may enhance locomotion by potentiation of psychostimulants effects, thereby indirectly increasing DA in the NAc. Nevertheless, enhanced DA and 5-HT following repeated METH is highly correlated with hyperlocomotion seen in both adult and developing rats.

Phencyclidine and Locomotor Activity. In adult rats, acute injection of phencyclidine (PCP) produces hyperlocomotion, stereotypy, and ataxia in a dose dependent manner (Sturgeon et al., 1979; Tani et al., 1994). In developing rats, PCP differentially affects locomotor activity depending on the age. On postnatal day 10 (PD 10), a low dose of PCP (1.5 mg/kg) enhanced locomotion, whereas a medium dose (3.0 mg/kg) failed to produce hyperlocomotion (Pamela et al., 2000). During PD 21-60, the same doses of PCP (1.5-3.0 mg/kg) increased locomotion in a dose dependent manner, with the greatest magnitude of change in locomotion in PD 21 rats (Pamela et al., 2000). Similarly, a low dose of PCP (1.0 mg/kg), compared to a moderate dose (4.0 mg/kg), produced the greatest locomotion on PD 12, whereas a dose-dependent increase in locomotion was found on PD 19 (Scalzo & Burge, 1994). Thus, unlike adult rats that

showed enhanced locomotion in a dose dependent manner, there is a distinctively different pattern in PCP effects on locomotion in developing rats. This pattern of responsiveness to PCP varies across different postnatal days, with a more variable pattern during early development and a less variable one during a later stage of development.

During withdrawal, adult rats treated with PCP (20-30 mg/kg/day, 6 days) showed a decrease in locomotor activity when tested 11 days after the last injection (Sams-Dodd, 2004). In developing rats, daily injection of PCP (7.5 mg/kg, once/day, 16 days) during PD 24-39 produced long-lasting hypolocomotion, whereas the same doses of PCP during PD 4-19 failed to affect locomotion (Scalzo, 1996). Thus, like METH, PCP produces a distinctively different pattern of locomotion in developing rats. This pattern of responsiveness to PCP varies across different postnatal days, with smaller changes in locomotion during early development and greater changes during later development.

Neurochemical Change Associated with PCP. PCP is a glutamate antagonist that blocks N-methyl-D-aspartate (NMDA) receptors in the brain (Zukin & Javitt, 1993). However, PCP-induced hyperlocomotion is thought to be due to enhanced dopamine (DA) and glutamate in the mesolimbic system, which includes the nucleus accumbens (NAc) and the prefrontal cortex (PFc). Direct infusion of PCP into the NAc produced hyperlocomotion and increased DA in the NAc (McCullough & Salamone, 1992), while depletion of DA in the NAc attenuated PCP-induced hyperlocomotion (Steinpreis & Salamone, 1993). Similarly, direct infusions of PCP into the PFc

produced locomotor enhancement, whereas neurotoxic lesions in the PFc attenuated the PCP-induced hyperlocomotion (Jentsch et al., 1998). Although these reports clearly suggest that PCP-induced hyperlocomotion is due to enhanced DA in the NAc and the PFc, the mechanism by which PCP produces hyperlocomotion is not well understood. One possible explanation is that PCP increases DA level in the NAc and the PFc by blocking NMDA receptors in the GABAergic interneurons within the ventral tegmental area (VTA). The VTA sends a major dopamine projection to the NAc and PFc. Thus, inhibition of inhibitory input to the VTA would lead to excitation of the VTA, which in turn would increase release of DA in the NAc and PFc (Wise, 2000). An alternative explanation is that PCP enhances glutamate transmission in the NAc and PFC, thereby producing hyperlocomotion. Microdialysis study showed a glutamate increase in the NAc and PFc after PCP treatment (Barbara & Moghaddam, 1998), and blockade of non-NMDA glutamate receptors in the NAc and the PFc inhibits PCP-induced hyperlocomotion without changing DA level (Takahata & Moghaddam, 2003). Although these reports provide evidence for critical involvement of glutamate in PCP-induced hyperlocomotion, it is unclear how PCP, a NMDA receptor antagonist, enhances glutamate transmission in the NAC and PFc. Nevertheless, enhanced levels of DA and glutamate within the mesolimbic system, particularly, the NAc and the PFc, are closely associated with PCP-induced hyperlocomotion.

Effects of Methamphetamine and PCP on Social Interaction: Short-term

Withdrawal Phase

In rats, social interaction is often defined by interaction between two 'unfamiliar' rats in a neutral arena (File & Seth, 2002; Tonissaar et al., 2004). Social interaction is typically measured by the time spent in active interaction, including sniffing, following (chasing), or grooming the partner during behavioral testing (File & Seth, 2002; Tonissaar et al. 2004). Social interaction is frequently employed to test the effect of pharmacological and surgical treatment on anxiety (See File & Seth, 2002).

Methamphetamine and Social Interaction. Previous studies have suggested that amphetamine derivatives, such as METH and 3,4-methylenedioxymethamphetamine (MDMA or ecstasy), affect social interaction (Clemens et al., 2004; McGregor et al., 2003). In adult rats, only moderate doses (5 mg/kg, 4 injections) of METH, but not low doses (2.5 mg/kg, 4 injections), decreased social interaction four weeks after injections (Clemens et al., 2004). Similarly, exposure to an amphetamine derivative during development appears to produce a long-lasting decrease in social interaction. For example, rats exposed to MDMA on PD 39 decreased social interaction 12 days after the last injection (Fone et al., 2002). Exposure to MDMA on PD28 also produced a long-term decrease in social interaction when tested during adulthood, PD 84 (Bull et al., 2004). Thus, unlike locomotor activity, exposure to METH at different stages of development would be expected to produce comparable deficits in social behavior. Given the evidence that METH and MDMA produce neurotoxic effects on the same brain regions (Armstrong & Noguchi, 2004), exposure to METH during the developmental period would be expected to produce a prolonged decrease in social

interaction.

Methamphetamine and Neural Structures Implicated in Social Interaction. The prefrontal cortex (PFc) has been implicated in mediation of social interaction. In rats, for example, the medial part of the prefrontal cortex is thought to mediate social interaction by regulating fear- and anxiety-related behavior (Gonzalez et al., 2000). Animal studies with PFc lesions, however, have yielded inconsistent results. For example, bilateral lesions in the medial prefrontal cortex both increased fear response (Gonzalez et al., 2004) and decreased fear response (Shah & Treit, 2003). According to Rangel et al. (2003), PFc lesions produced an anxiolytic effect the second week after the surgery, and they produced anxiogenic effect fifth week post-surgery, suggesting that the state of anxiety depends on a progressive change in the prefrontal cortex. Presumably, the behavioral shift from a 'less anxious state' to a more anxious state' would be reflected in social interaction during the course of change in the prefrontal cortex.

Repeated administration of high doses of METH produce neurotoxic effects on dopaminergic and serotonergic axon terminals (Armstrong & Noguchi, 2004; Frost & Cadet, 2000) and produce persistent depletion of DA and 5-HT in various brain regions. In particular, neurotoxic doses of METH deplete DA in the striatum and the PFc (Clemens et al., 2004; Wagner et al., 1980). Parallel studies have demonstrated that neurotoxic doses of METH deplete 5-HT in the hippocampus, the PFc, the striatum, and the amygdala (Armstrong & Noguchi, 2004; Daberkow et al., 2005; Schroder et al., 2003; Wrona, et al., 1997). Given the evidence that reduction of 5-HT

level in these brain regions was highly correlated with a long-term decrease in social interaction following MDMA (ecstasy) administration (Mcgregor et al., 2005), impaired social interaction appears rather specific to reduced 5-HT function in these brain regions.

Phencyclidine and Social Interaction. Few studies have investigated PCP effects on social interaction in adult rats. To my knowledge no study has investigated PCP effects on social interaction in developing rats. Sams-Dodd (1996, 1998) reported that in adult rats, either single injections or continuous administration of PCP via mini pumps reduced social interaction. One study examined the effect of PCP on social interaction during the withdrawal period. According to Sams-Dodd (2004), social interaction tested 10 days after PCP injection (5-30 mg/kg/day for 6 consecutive days) at 3 months of age was not affected. Moreover, coadministration of a neurotoxic dose of PCP (50 mg/kg, one or four injections) in conjunction with pilocarpine, which promotes PCP-induced neurotoxicity, given at 4 months of age also failed to affect social interaction which was tested on day 10 of withdrawal (Sams-Dodd, 2004). Sams-Dodd's findings suggest that in adult rats social interaction is affected by PCP acutely but restored during the withdrawal period. In developing rats, the effects of PCP on social interaction during the withdrawal period are unknown. Further studies are needed to examine the effect of PCP on social interaction in developing rats.

Phencyclidine and Neuronal Structures Implicated in Social Interaction. PCP, like METH, produces neurotoxicity in the brain. A Single dose of PCP (5 mg/kg) changed

gene expression in the prefrontal cortex, and such change in expression of transcripts was thought to be associated with the immediate toxic effects of PCP (Kaiser et al., 2004). In adult rats, continuous infusion of PCP (5.45 mg/kg/day) across 5 days increased glucose metabolism in the limbic system and cortical regions 24 hr and 10 days after the treatment (Elllison et al., 1996). These regions included the hippocampus, the retrosplenial cortex, and the posterior cingulate cortex. The authors hypothesized that the persistent increase in glucose metabolism in these structures was due to widespread neurotoxicity during the withdrawal period. Thus, although behavioral evidence with respect to changes in social interaction is insufficient, the dysfunctional state of cortical and limbic structures, such as the hippocampus, may impair social interaction.

Effects of Methamphetamine and PCP on Spatial Learning: Long-term Withdrawal Phase

Methamphetamine and Spatial Learning. In adult rats, acute injection of neurotoxic doses of METH produces long-term deficits in learning and memory. METH-treated rats (four injections of 12.5 mg/kg at 2 hr interval) showed impaired learning when they were tested in the spatial watermaze task 65 days after the treatment (Friedman et al., 1998), suggesting that spatial learning was impaired by METH. More recent reports indicate that METH injections (4 mg/kg, 4 injections at a 2 hr interval) impaired short- and long-term memory in a novel object recognition task when testing occurred 1 and 3 weeks after the treatment, without affecting the acquisition and

retention of spatial information (Schroder et al., 2003). Similarly, following four days of withdrawal, METH (three injections of 10 mg/kg at a 2 hr interval) produced a selective memory deficit in the novel object recognition task, but not in the spatial version of the recognition task, which involved simply moving the sample object to a new location (Bisagno et al., 2002).

In developing rats, repeated administration of METH affects learning if it is given within a specific period during development. According to Vorhees at al. (1994), repeated injections of neurotoxic doses of METH (30 mg/kg, twice/day, 10 days) during PD 11-20 impaired the acquisition of a spatial water maze task tested on approximately PD 50 (Vorhees et al., 1994). On the other hand, the same dose and frequency of METH injections failed to affect acquisition if it was given during PD 1-10 (Vorhees et al., 1994), suggesting that exposure to METH during the later stage, not the earlier stage, of development is detrimental to new learning. Subsequent studies by the same group examined METH effects on learning using a lower dose and a greater number of injections than those used in their previous study (10 mg/kg/day at 2 hr interval, 4 injections/day, 10 days). They found that METH exposure during PD 11-20 impaired spatial and reversal learning when tested around PD 50 (Vorhees et al., 2000). Interestingly, the same dose and frequency of METH injections selectively impaired the acquisition of spatial reference memory and the reversal task, without affecting spatial working memory (Williams et al., 2003a). Moreover, when METH was given (10 mg/kg, 4 injections/day at a 2 hr interval, 10 days) during PD 11-15, performance in the spatial reference and reversal task was

impaired, whereas METH given during PD 15-20 failed to have these effects (Williams et al., 2003b). More recent study by the same group examined the effects of METH on learning following exposure to different doses at various stages, PD 21-30 (2.5-10 mg/kg), PD 31-40 (1.25-7.5 mg/kg), PD 41-50 (1.25-5.0 mg/kg), and PD 51-60 (1.25-5.0 mg/kg). The justification for using various doses was that rate depended on developmental stage: the highest dose used in their previous reports (10mg/kg, 4 injections/day, 10 days) is known to produce toxicity. When the rats were tested in a learning task in adulthood, only the rats exposed to METH during PD 41-50 showed impaired spatial reference memory (Vorhees et al., 2005). These findings indicate that during development there is a specific time-window when the nervous system is sensitive and vulnerable to neurotoxic doses of METH. Clearly, exposure to METH at specific developmental periods produces an enduring learning deficit in adulthood.

Phencyclidine and Spatial Learning. Previous studies have demonstrated that in adult rats PCP and other non-competitive NMDA antagonists impair spatial learning during the acute drug state, and that learning was restored during the early withdrawal period (Campbell et al., 2004; Kesner & Dakis, 1993; Whishaw & Auer, 1989). Thus, the effects of a brief exposure to PCP on learning are transient rather than long-term in adult rats. On the other hand, chronic treatment with PCP impairs the acquisition of cognitive tasks, particularly set-shifting in adult rats. When the rats were treated PCP (5 mg/kg, 2 injections/day, 7 days) and tested after 7 days of withdrawal, PCP-treated rats showed a retarded acquisition in reversal of a visual discrimination task. However, the same animals showed normal acquisition in a novel visual discrimination

(Jentisch & Taylor, 2001). The authors attributed the selective deficit in the reversal learning to impairment in inhibitory control (Jentsch & Taylor, 2001). Thus, chronic administration of PCP may impair flexibility in set-shifting, particularly intra-dimensional shift, where the discriminative stimuli in the same dimension are switched (Dalley et al., 2004; Jentsch & Taylor 2001). Similarly, compared to controls, PCP-treated rats (the same as those used in Jentisch & Taylor) required a greater number of trials to reach a behavioral criterion when the rule was shifted extra-dimensionally (Rodefer et al., 2005). Thus, chronic administration of PCP appears to produce inflexibility in set-shifting when the discriminative stimuli in one dimension (odor) are switched to the other (medium) (Rodefer et al., 2005). Thus, in adult rats, chronic, but not acute, administration of PCP selectively affects the ability to shift context-appropriate rules.

In developing rats, exposure to PCP during development produces a long-lasting effect on spatial learning in young adulthood. Daily injection of PCP (5 mg/kg/day) during PD 5-15 disrupted the acquisition of spatial water maze task tested on PD 35 and PD 60 (Sircar, 2003). In addition, rats treated with PCP (8.7 mg/kg) on PD 7, 9 and 11 showed an impaired acquisition of spatial reference, reversal and working memory tasks tested in adulthood (Andersen & Pouzet, 2004). Thus, it appears that early exposure to PCP produces profound effects on learning in adulthood. Unlike METH, which required a time-window of sensitivity at a specific developmental period to produce an enduring learning deficit, exposure to PCP from as early as PD 5-10 produces enduring effects on cognitive behavior in adulthood.

Rationale for METH and PCP Treatment on PD 50-51. Although previous studies have indicated that exposure to METH and PCP during an early development produces behavioral deficits (Sircar, 2003; Wallance et al., 2003b), the effects of exposure to METH and PCP at a later developmental stage (PD 50-51) on a range of behavior have not been investigated. According to Vorhees et al. (2005), METH treatment during PD 41-50 impaired spatial learning tested in adulthood. To my knowledge, no study has investigated the effects of PCP exposure during late development and its long-term behavioral consequences. Given the evidence that NMDA receptor antagonists, including PCP, begin to produce neurotoxicity in the limbic structures on PD 45 (Farber et al. 1995; Farber, 2003), exposure to PCP after PD45 would produce long-term behavioral deficits that are differentiated from the other developmental stages.

METH and PCP also affect the dopaminergic system in the prefrontal cortex and the striatum (Jentsh et al., 1998; Shoblock et al., 2003; White et al., 1995). Thus, exposure to these drugs during development would affect development of the dopaminergic system and lead to long-term behavioral deficits. In rats, the dopaminergic systems mature during PD 40-60 by increasing the density of dopamine receptors in the PFc and the striatum until PD 60 (Kalsbeek et al., 1988). In particular, the density of prefrontal DA receptors peaks around PD 40-60 (Anderson et al., 2000), whereas the density of DA receptors in the striatum peaked around PD 40 and declined until PD 120 (Gelbard et al., 1989), suggesting that METH and PCP exposure during later development would affect the dopaminergic system.

Clearly, these studies indicate that exposure of METH and PCP during the late development (PD 50-51) would affect both dopaminergic and glutamatergic systems in the prefrontal cortex and the striatum during development, and exposure to these drugs are likely to produce long-term behavioral deficits.

Specific Aims of the Thesis

The present study was aimed to investigate the acute and long-term effects of exposure to high doses of METH and PCP on locomotor activity, social interaction, and spatial and reversal learning. Juvenile rats (PD 50-51) were treated with METH (9 mg/kg) or PCP (9 mg/kg), twice per day at a 12 hr interval for two consecutive days. Locomotor activity was measured during acute and withdrawal periods (PD 50-79), social interaction was measured during the withdrawal period (PD 54-79), and spatial and reversal learning were tested in adulthood (PD 90 or older) (see Table. 1). Hypotheses were: (1) Juvenile rats exposed to METH and PCP will increase locomotor activity at the acute stage and decrease locomotor activity during the withdrawal stage; (2) METH- and PCP-treated rats will decrease social interaction during the withdrawal stage; (3) Rats exposed to METH and PCP during PD 50-51 will show deficits in spatial and reversal learning in adulthood.

Methods

Experiment 1. Effects of Methamphetamine and PCP on Locomotor Activity:

Table 1

Schedule of Treatment and Behavioral Measurement

TREATMENT / BEHAVIOR	ACUTE STATE				WITHDRAWAL STATE				LONG-TERM
	FIRST INJECTION	SECOND INJECTION	THIRD INJECTION	FOURTH INJECTION	DAY 3	DAY 7	DAY 14	DAY 28	POSTNATAL DAY 90 or older
LOCOMOTOR ACTIVITY	←		→ ←		Short-term →		Long-term →		
SOCIAL INTERACTION					Short-term →		Long-term →		
SPATIAL LEARNING									← →

Note. Locomotor activity, social interaction, and spatial learning were measured at different periods. The first injection was on postnatal day 50. Locomotor activity was measured twice during the acute drug state, and four times during withdrawal. Social interaction was measured 4 times during withdrawal. Spatial learning was measured after animals reached adulthood, postnatal day 90 and after (long-term withdrawal). Arrows (← →) indicate behavioral testing period.

Acute Drug Phase and Withdrawal Phase

Subject

Thirty-two Wistar rats (postnatal day 30-79; 150-200 g at start of the experiment) were used in this experiment and treated in accordance with NIH guideline. Rats were housed in the Psychology Department Laboratory at Morehead State University under a 12/12 light-dark cycle (10:00/22:00), and food and water were available *ad libitum*. Rats were housed in gang cages (4 rats/cage) to maintain a comparable environment to that of the social interaction experiment. All animals were handled for 5 min per day for at least 3 days prior to the beginning of the experiment.

Apparatus

Locomotor activity was measured in an open-field arena, which contained four zones in a square. A video camera mounted on the ceiling of the room and centered above the field could monitor the activity of a subject in each zone of the field. Output from the video camera was routed to a VCR, which sent the output to a computer. Real-time activity was shown on a monitor, and a video tracking system collected and quantified locomotion, using the contrast between the light subject (rat) and the dark background at a speed of 30 images/sec. Computer software analyzed distance traveled every 5 min for a 60 min period. The room was illuminated by two standing lights (150 Watts/ light) beside the open-field and one lamp (25 Watts) positioned above the video camera.

Locomotor Activity

Animals were separated from the group and placed into one of four zones with the

treatment conditions randomized. Locomotor activity was measured for a 60 min period. After each session, animals were returned to their gang cage.

Drug Administration

On postnatal day (PD) 50-51, a total of four injections of METH (9.0 mg/kg, s.c.), PCP (9.0 mg/kg, s.c.) or saline (0.9%, 1 ml/kg, s.c.) were administered at a 12-h interval (8:00 am, 8:00 pm). Rats were divided into two groups: METH and PCP. In the METH group (n = 16), two of four rats received METH injections, and the remaining two received saline. Similarly, in the PCP group (n = 16), two of four rats received PCP injections, and the remaining two received saline.

Experimental Design

One day prior to the first injection, animals were habituated to the open-field for 60 min. The acute effect of the treatment was measured immediately after the first (day 1) and the third (day 2) injection. The withdrawal effect of the treatment was measured during a short-term (3 days and 7 days after the last injection, PD 54 and 58) and a long-term period (14 and 28 days after the last injection, PD 65 and 79).

Data Analysis

Locomotor activity of drug-treated vs. vehicle-treated rats was analyzed with two-way mixed ANOVA: separate analyses were done for acute (3 treatments x 2 sessions) and withdrawal effects (3 treatments x 4 sessions). In addition, each session was further analyzed by two-way mixed ANOVA (3 treatments x 12 five-min bins). LSD procedure was employed for post hoc analysis. Vehicle-treated rats from the METH and PCP groups were combined and treated as one vehicle group because

two-way ANOVAs yielded no significant difference between two vehicle groups during the acute and the withdrawal states. One vehicle animal was excluded from the analysis due to a tracking problem.

Experiment 2: Effects of Methamphetamine and PCP on Social Interaction

Subjects

Fifty-six Wistar rats (PD 30-79) were used in this experiment. On PD 30, animals were divided into groups of four (total 14 groups). Each subject in the group was color coded using permanent markers: red for drug-treated through the dorsal surface, black for saline control at the posterior part of the dorsal surface, green for one vehicle control at the center part of the dorsal surface, and yellow for the other vehicle control at the anterior part of the dorsal surface.

Social Interaction

Social interaction of four rats was measured in the open-field (4 rats/compartment). The interaction was taped by a video camera mounted on the ceiling for a 60 min period for off-line analysis. Social interaction was defined by two criteria: 1) active approach to other rats and 2) turning the head toward another rat approximately 45 degrees or greater and touching the other rat's body. Specifically, the frequency and the duration of each criterion were measured. The 60-min observation period was divided into 7 segments (8 min/segment), excluding the first 2 min, which was a brief habituation period in each session. During each segment (8 min), social interaction of saline- and drug-treated rats was scored separately, by alternating 4 min intervals

within each 8-min segment. Frequency of the criteria was ranked in a range of five (0-4): 0 = 0; 1 = 1; 2-5 = 2; 6–13 = 3; > 13 = 4. Duration of the criteria was also ranked in a range of five (0-4): 0 sec = 0; 1-86 sec = 1; 87–159 sec =2; 160-239 sec = 3; >239 sec = 4). This range of scoring system was based on a distribution of frequency and duration of criteria every 25%. Social interaction score was obtained by multiplying frequency and duration scores. An overall social interaction score was obtained by adding the scores of 7 8-min segments. This scoring system is similar to methods used in previous studies (White et al., 1998; Fone et al., 2002). Frequency of contacts was positively skewed and duration of contacts was negatively skewed. The distribution of multiplied scores (Frequency x Duration) yielded a standard normal curve. Pearson correlation showed 96% intraobserver reliability in the frequency measure and 99% intraobserver reliability in the duration measure.

Drug Administration

On postnatal days 50-51, a total of four injections of METH (9 mg/kg, s.c.), PCP (9 mg/kg, s.c.) or saline (0.9%, 1ml/kg, s.c.) were administered at a 12-h interval (8:00 am, 8:00 pm). Rats were divided into two groups: METH and PCP. In the METH group (n = 28), two of four rats from a gang cage received treatment: one METH (n = 7), one saline (n = 7). The remaining two did not receive any treatment (n = 14). Similarly, in the PCP group (n = 28), two of four rats from a gang cage received treatment: one received PCP (n = 7), one received saline (n = 7). The remaining two did not receive any treatment (n =14).

Experimental Design

Animals were habituated to the open-field for 60 min prior to the experiments. Social interaction was measured during the withdrawal period, on Day 3, 7, 14 and 28 after the last injection.

Data Analysis

Social interaction of the METH group and the PCP group was analyzed separately. Two-way mixed ANOVA (2 treatments x 4 sessions) was used to analyze social interaction. Data were further analyzed by LSD post hoc analysis. One PCP-treated animal was sick and was eliminated after the third injection (n = 6 per treatment in the PCP group).

Experiment 3: Effects of Methamphetamine and PCP on Spatial Discrimination and Reversal

Subjects

Thirty-four Wistar rats treated with METH (n=8), PCP (n=8), or saline (n=18) on PD 50-51 were used in adulthood (PD 90 and after) in this study. Rats were separated from the group and housed in ingle cages. All animals were handled for 5 min per day for 3 consecutive days. Food was restricted to keep a subject at least at 85% of its original weight and to train rats on the learning task.

Apparatus

Eight operant chambers (29.4 cm W x 24.5 cm D x 29.4 cm H) were used in this experiment. Each chamber was equipped with a house light, two retractable bars, two

signal windows (red and yellow cues), a speaker, and a pellet dispenser located between the levers. Each chamber was placed in a sound-attenuating box (75.95 cm W x 51.45 cm D x 51.45 cm H).

Behavioral Tasks

Initially the animals were shaped to barpress in the operant chamber. Shaping included three steps: hopper training, barpress training, and position-bias removal. In hopper training, food reward was associated with the illuminated hopper. In barpress training, animals were successively shaped to barpress: first, the animals were rewarded when approaching the lever; then they had to put their paw on the lever; finally, they received food only when they pressed lever. In position-bias removal, animals were trained press both right and left levers equally often.

Following bar-press training, animals were trained in the spatial discrimination task (SD), which required a barpress opposite to the cue location. For example, an animal had to press a right lever in response to a left light cue regardless of the color (red or yellow) (Fig. 1A). The animals were given 2 sec to respond following cue illumination, and the cue was turned off immediately after a correct or incorrect response, or after 2 sec elapsed. Trial types were presented in a pseudorandom fashion. An incorrect response produced a brief tone (95 dB, 500 msec) and terminated the trial. The inter-trial interval (ITI) was 8 sec. Premature barpresses prior to the onset of the stimulus reset the trial. A training session was terminated either when the animal consumed 100 pellets (Noyes, 45 mg) or after 60 min elapsed. A computer collected the percent correct responses, response latencies

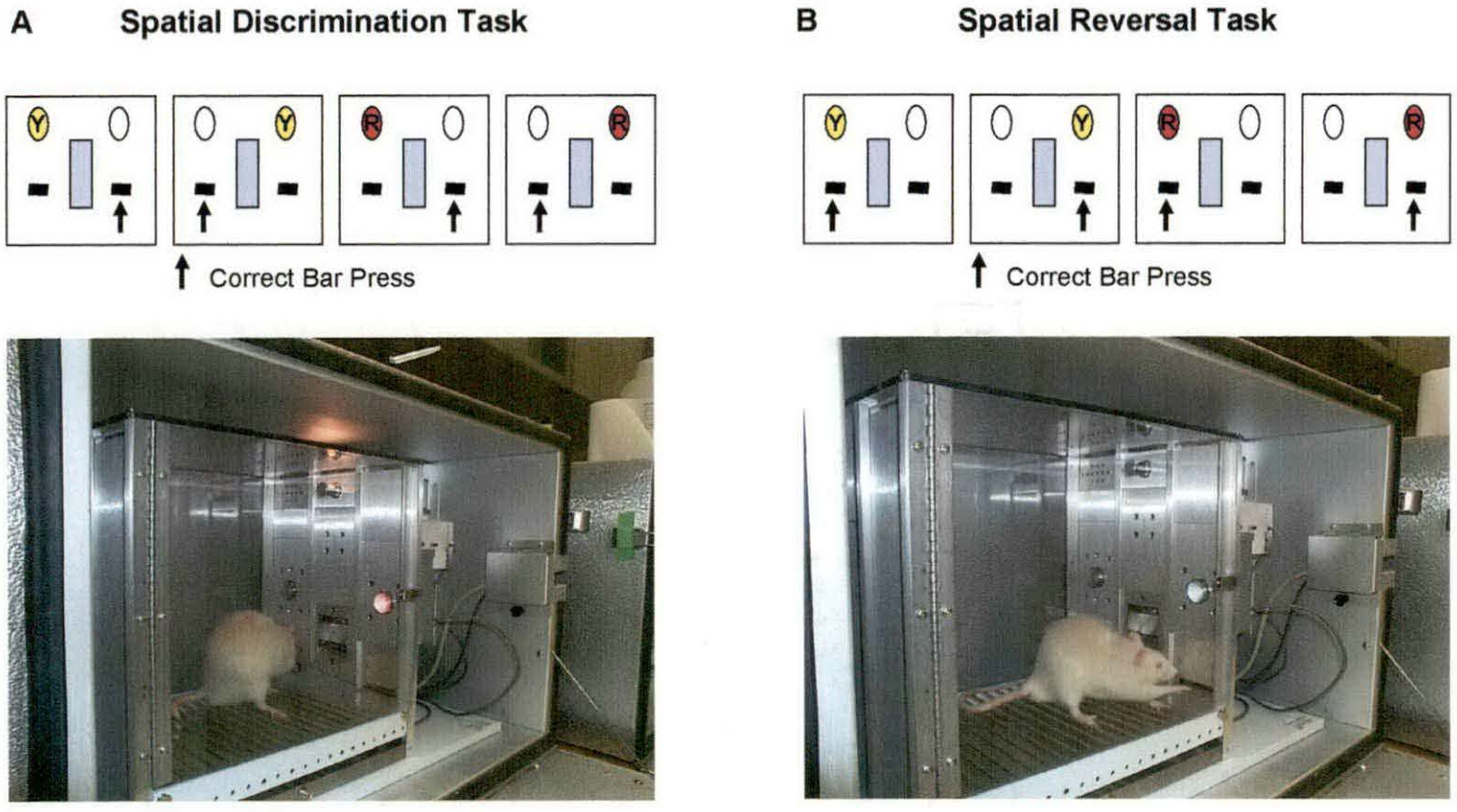


Figure 1. Spatial discrimination task and reversal task. Stimulus cue was presented either at right or left with two different colors (red or yellow) at each trial. In spatial discrimination task **(A)**, rats required a non-matching barpress to the cue location. In reversal task **(B)**, rats required a matching barpress to the cue location. The arrow indicates the correct response.

(correct and incorrect), and the number of barpresses during the ITI For acquisition, a behavioral criterion (≥ 85% correct responses, three consecutive days) was used.

Reversal training began when the animal reached the behavioral criterion on SD. In the spatial reversal task (SDR), a correct response was defined as a barpress same as the cue location. Thus, the animals had to press a right lever in response to a right stimulus cue, *vice versa* (Fig. 1B). The other conditions were exactly identical to those for the SD, and the same variables were collected during the acquisition of SDR. The behavioral criterion for acquisition of the reversal task was ≥ 85 % correct responses for three consecutive days.

Drug Administration

Four injections of methamphetamine (9 mg/kg s.c.) or phencyclidine (9 mg/kg s.c.) were administered on PD 50-51. Each injection was given every 12 hours (8:00 am, 8:00 pm). Control subjects received saline injections on the same schedule.

Experimental Design

Animals were divided into two groups: METH (n = 8) and PCP (n = 8) with paired vehicles (n = 9 in each group). The METH group was divided into two squads, and each squad had four METH-treated and four saline-treated rats. Similarly, the PCP group was divided into two squads with four PCP-treated and four saline-treated rats in each squad. Two saline-treated rats were trained separately. In the first squad, drug-treated rats were assigned to chambers 1-4, and saline-treated rats were assigned to chambers 5-8, whereas, in the second squad, drug-treated rats were assigned to chambers 5-8 and saline-treated rats were assigned to chambers 1-4. Rats were

trained in the task once a day. SD lasted until the animal reached the behavioral criterion, whereas SDR lasted for 22 days.

Data Analysis

Statistical analyses were performed separately in the METH group and the PCP group. Two-way mixed ANOVA (2 treatments x 22 training sessions per task) was employed to analyze the percent correct responses, response latencies (correct and incorrect), and number of barpresses during the ITI. LSD pair-wise comparisons were performed for further analyses. One-way between-subject ANOVA was used to analyze the number of training days required to reach the criterion in each treatment group. During the acquisition of SD, some rats reached the behavioral criterion in the earlier training phase. Their training was terminated at that point because over-training in SD could confound the acquisition of SDR. For the statistical analysis the missing values for remaining sessions in SD were replaced with the value of the last session.

Results

Experiment 1: Methamphetamine and PCP on Locomotor Activity: Acute and Withdrawal (short- and long-term)

Locomotor activity was measured twice during the acute drug state, immediately after the first injection (PD 50) and the third injection (PD 51), and it was measured four times during the withdrawal period: 3 days (PD 54), 7 days (PD 58), 14 days (PD 65) and 28 days (PD 79). Overall locomotion during a 60 min session was compared

among three treatments in acute (3 x 2 ANOVA) and withdrawal periods (3 x 4 ANOVA). Behavioral activity was further analyzed every 5 min across a 60 min period (3 x 12 ANOVA).

Acute Drug State. Immediately after the first injection, METH- and PCP-treated rats showed enhanced locomotion compared to saline-treated rats. Immediately after the third injection, however, METH-treated rats showed locomotor activity comparable to that of saline-treated rats, whereas PCP-treated rats showed a further enhanced locomotion (Fig. 2). A 3 x 2 ANOVA yielded a significant treatment effect [F (1, 28) = 23.15, $p < .001$] and interaction between treatment and injection [F (2, 28) = 43.61, $p < .001$], but not a significant effect of injection [F (1, 28) = 0.02, $p > .05$]. For the first injection, the post hoc analysis revealed a significant difference between METH and saline treatment [t (21) = 4.50, $p < .001$], and PCP and saline treatment [t (21) = 2.39, $p < .05$]. At the third injection, the post hoc analysis revealed a significant difference between PCP and saline treatment [t (21) = 9.75, $p < .001$], but not METH and saline treatment [t (21) = -0.35, $p > .05$].

Immediately after the first injection, METH-treated rats increased locomotor activity during the initial 25 min period, whereas PCP-treated rats increased locomotor activity during the last 25 min, compared with saline-treated rats (Fig. 3A). Immediately after the third injection, METH-treated rats showed locomotor activity comparable to that of saline-treated rats, whereas PCP-treated animals showed enhanced locomotion throughout the 60 min period (Fig. 3B). A 3 x 12 ANOVA on the first injection yielded a significant treatment effect [F (2, 28) = 10.53, $p < .001$],

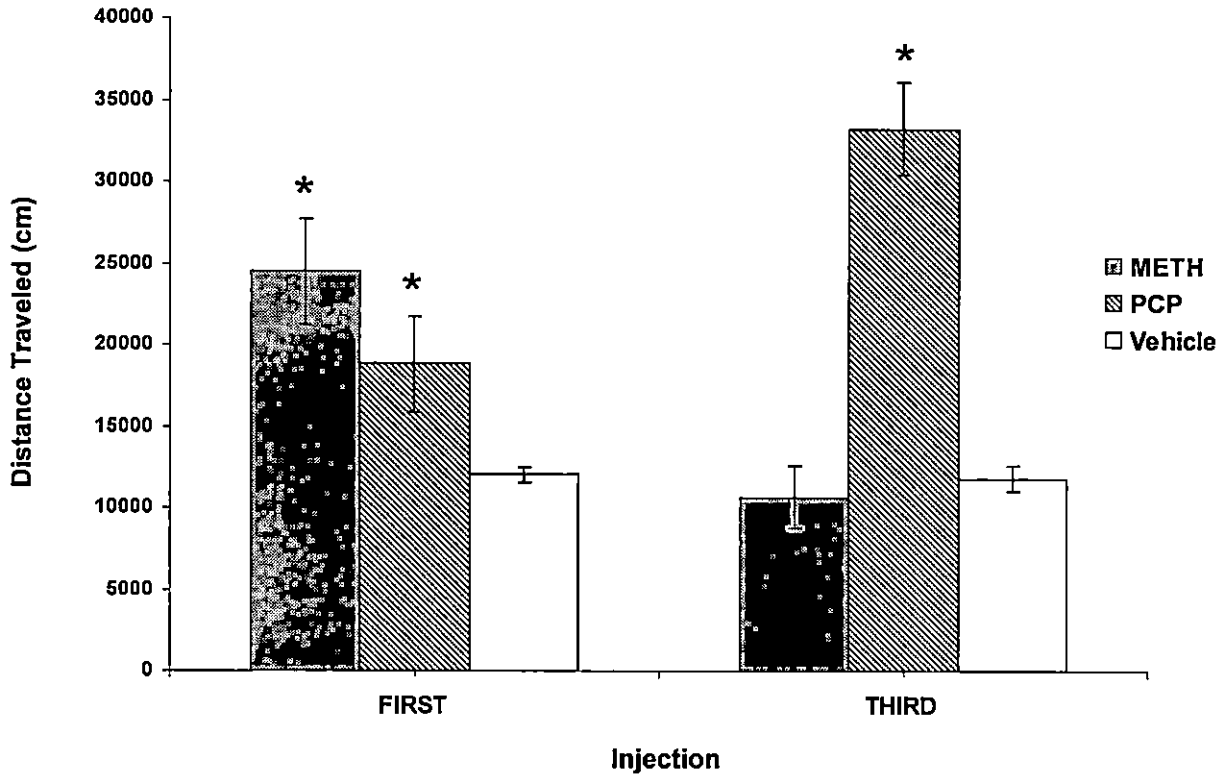


Figure 2. Acute effects of methamphetamine (METH) and phencyclidine (PCP) on total locomotion (60 min). The horizontal axis represents the order of injection. The vertical axis represents the distance traveled (cm) during the 60 min period. Error bars indicate standard error of the mean (SEM). Immediately after the first injection, both METH and PCP enhanced locomotion. Immediately after the third injection, METH did not affect locomotor activity, while PCP further enhanced locomotion. $*p < .05$.

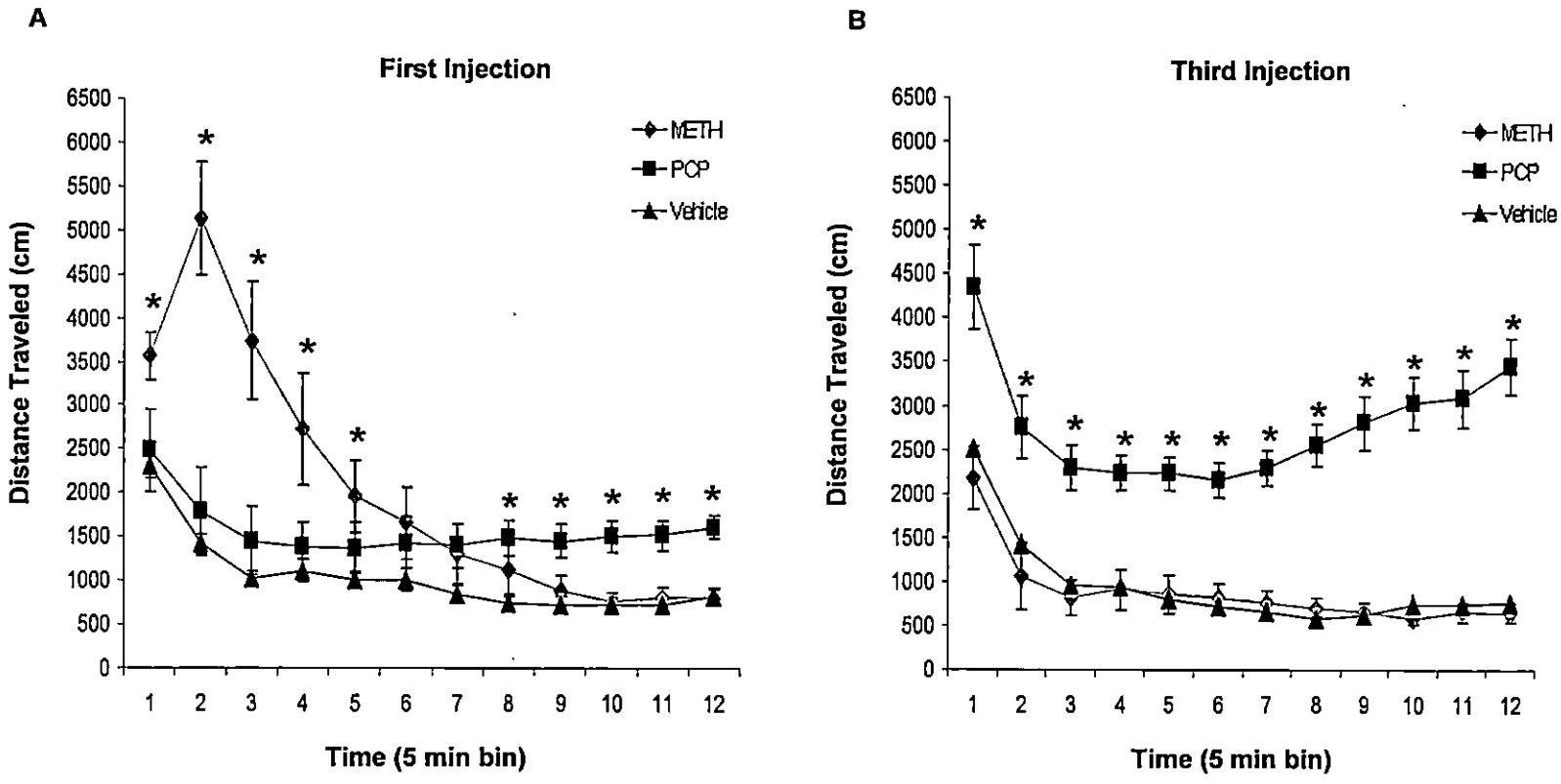


Figure 3. Effects of the first and the third injection of methamphetamine (METH) and phencyclidine (PCP) on locomotor activity. The horizontal axis represents the time (5 min bin) and the vertical axis represents distance traveled (cm). Error bars indicate SEM. Immediately after the first injection **(A)**, METH produced hyperlocomotion during the first 25 min and decreased during the remaining period, while PCP did not affect locomotion during the first 35 min, but gradually increased locomotion during the last 25 min. Immediately after the third injection **(B)**, METH did not affect locomotor activity throughout 60 min period while PCP further enhanced locomotion throughout 60 min period. $*p < .05$

time passage effects [F (11, 308) = 34.96, p < .001], and interaction between treatment and time passage [F (22, 308) = 12.88, p < .001]. The following post hoc pair-wise comparison revealed a significant difference between METH and saline treatment for the initial 25 min and between PCP and saline treatment for the last 25 min (see Table. 2). A 3 x 12 ANOVA for the third injection yielded a significant treatment effect [F (2, 28) = 55.53, p < .001], time passage effects [F (11, 308) = 43.67, p < .001], and interaction between treatment and time passage [F (22, 308) = 4.81, p < .001]. The following post hoc pair-wise comparison revealed a significant difference between PCP and saline treatment throughout the 60 min period, whereas METH and saline groups did not differ throughout the 60 min (see Table. 3).

Withdrawal State. During the withdrawal period, drug-treated rats (METH and PCP) showed locomotor activity comparable to that of saline-treated rats (Fig. 4). Animals across all the treatments increased locomotor activity as days progressed up to 14 days after the last injection. However, this tendency was not found 28 days after the last injection. A 3 x 4 ANOVA yielded a significant effect of days after the last injection [F (3, 84) = 10.75, p < .001], but did not show a significant treatment effect [F (2, 28) = 0.72, p > .05], and a significant interaction between treatment and days [F (6, 84) = 1.81, p > .05].

On Day 3 after the last injection, METH-treated rats decreased locomotion during the initial 5 min and increased locomotion between 25 and 30 min, compared to saline-treated animals. On the other hand, PCP-treated rats showed locomotor activity comparable to that of saline-treated rats (Fig. 5A). On Days 7, 14, and 28

Table 2

Effects of the First Injection of METH and PCP on Locomotor Activity

Time	Treatment		Mean Diff	Std. Error	*p*-value	*t*-value
5 min	METH	Vehicle	1256.62	373.83	p < .01	3.36
	PCP	Vehicle	172.03	373.83	p > .05	0.46
	METH	PCP	1084.59	426.94	p < .05	2.54
10 min	METH	Vehicle	3718.16	518.83	p < .001	7.17
	PCP	Vehicle	358.42	518.83	p > .05	0.69
	METH	PCP	3359.74	592.54	p < .001	5.67
15 min	METH	Vehicle	2710.41	485.68	p < .001	5.58
	PCP	Vehicle	425.76	485.68	p > .05	0.88
	METH	PCP	2284.65	554.69	p < .001	4.12
20 min	METH	Vehicle	1610.32	461.82	p < .01	3.49
	PCP	Vehicle	264.16	461.82	p > .05	0.57
	METH	PCP	1346.17	527.44	p < .05	2.55
25 min	METH	Vehicle	954.45	336.19	p < .01	2.84
	PCP	Vehicle	363.07	336.19	p > .05	1.08
	METH	PCP	591.38	383.96	p > .05	1.54
30 min	METH	Vehicle	652.90	348.47	p > .05	1.87
	PCP	Vehicle	420.35	348.47	p > .05	1.21
	METH	PCP	232.55	397.98	p > .05	0.58
35 min	METH	Vehicle	456.00	285.60	p > .05	1.60
	PCP	Vehicle	548.22	285.60	p > .05	1.92
	METH	PCP	-92.21	326.18	p > .05	-0.28
40 min	METH	Vehicle	384.83	235.18	p > .05	1.64
	PCP	Vehicle	734.79	235.18	p < .01	3.12
	METH	PCP	-349.96	268.60	p > .05	-1.30
45 min	METH	Vehicle	169.14	202.81	p > .05	0.83
	PCP	Vehicle	735.93	202.81	p < .01	3.63
	METH	PCP	-566.80	231.63	p < .05	-2.45
50 min	METH	Vehicle	39.47	151.41	p > .05	0.26
	PCP	Vehicle	773.67	151.41	p < .001	5.11
	METH	PCP	-734.20	172.93	p < .001	-4.25
55 min	METH	Vehicle	78.80	153.09	p > .05	0.51
	PCP	Vehicle	788.20	153.09	p < .001	5.15
	METH	PCP	-709.39	174.84	p < .001	-4.06
60 min	METH	Vehicle	-17.23	152.63	p > .05	-0.11
	PCP	Vehicle	786.87	152.63	p < .001	5.16
	METH	PCP	-804.10	174.32	p < .001	-4.61

Note. Pair-wise comparisons were done with LSD for 5-min bin.

Table 3

Effects of the Third Injection of METH and PCP on Locomotor Activity

Time	Treatment		Mean Diff	Std. Error	*p*-value	*t*-value
5 min	METH	Vehicle	-336.04	387.64	p > .05	-0.87
	PCP	Vehicle	1827.24	387.64	p < .001	4.71
	METH	PCP	-2163.28	442.71	p < .001	-4.89
10 min	METH	Vehicle	-354.30	349.46	p > .05	-1.01
	PCP	Vehicle	1334.87	349.46	p < .001	3.82
	METH	PCP	-1689.17	399.11	p < .001	-4.23
15 min	METH	Vehicle	-132.26	240.95	p > .05	-0.55
	PCP	Vehicle	1344.76	240.95	p < .001	5.58
	METH	PCP	-1477.02	275.18	p < .001	-5.37
20 min	METH	Vehicle	-29.51	246.15	p > .05	-0.12
	PCP	Vehicle	1303.57	246.15	p < .001	5.30
	METH	PCP	-1333.07	281.13	p < .001	-4.74
25min	METH	Vehicle	61.57	205.48	p > .05	0.30
	PCP	Vehicle	1435.15	205.48	p < .001	6.98
	METH	PCP	-1373.58	234.67	p < .001	-5.85
30 min	METH	Vehicle	87.22	187.93	p > .05	0.46
	PCP	Vehicle	1427.22	187.93	p < .001	7.59
	METH	PCP	-1340.00	214.63	p < .001	-6.24
35 min	METH	Vehicle	103.09	172.33	p > .05	0.60
	PCP	Vehicle	1634.03	172.33	p < .001	9.48
	METH	PCP	-1530.94	196.82	p < .001	-7.78
40 min	METH	Vehicle	127.28	172.19	p > .05	0.74
	PCP	Vehicle	1969.15	172.19	p < .001	11.44
	METH	PCP	-1841.87	196.66	p < .001	-9.37
45 min	METH	Vehicle	30.91	218.22	p > .05	0.14
	PCP	Vehicle	2177.70	218.22	p < .001	9.98
	METH	PCP	-2146.79	249.22	p < .001	-8.61
50 min	METH	Vehicle	-162.50	234.51	p > .05	-0.69
	PCP	Vehicle	2295.62	234.51	p < .001	9.79
	METH	PCP	-2458.12	267.83	p < .001	-9.18
55 min	METH	Vehicle	-83.68	244.03	p > .05	-0.34
	PCP	Vehicle	2350.44	244.03	p < .001	9.63
	METH	PCP	-2434.12	278.70	p < .001	-8.73
60 min	METH	Vehicle	-104.39	234.11	p > .05	-0.45
	PCP	Vehicle	2692.02	234.11	p < .001	11.50
	METH	PCP	-2796.42	267.38	p < .001	-10.46

Note. Pair-wise comparisons were done with LSD for 5-min bin.

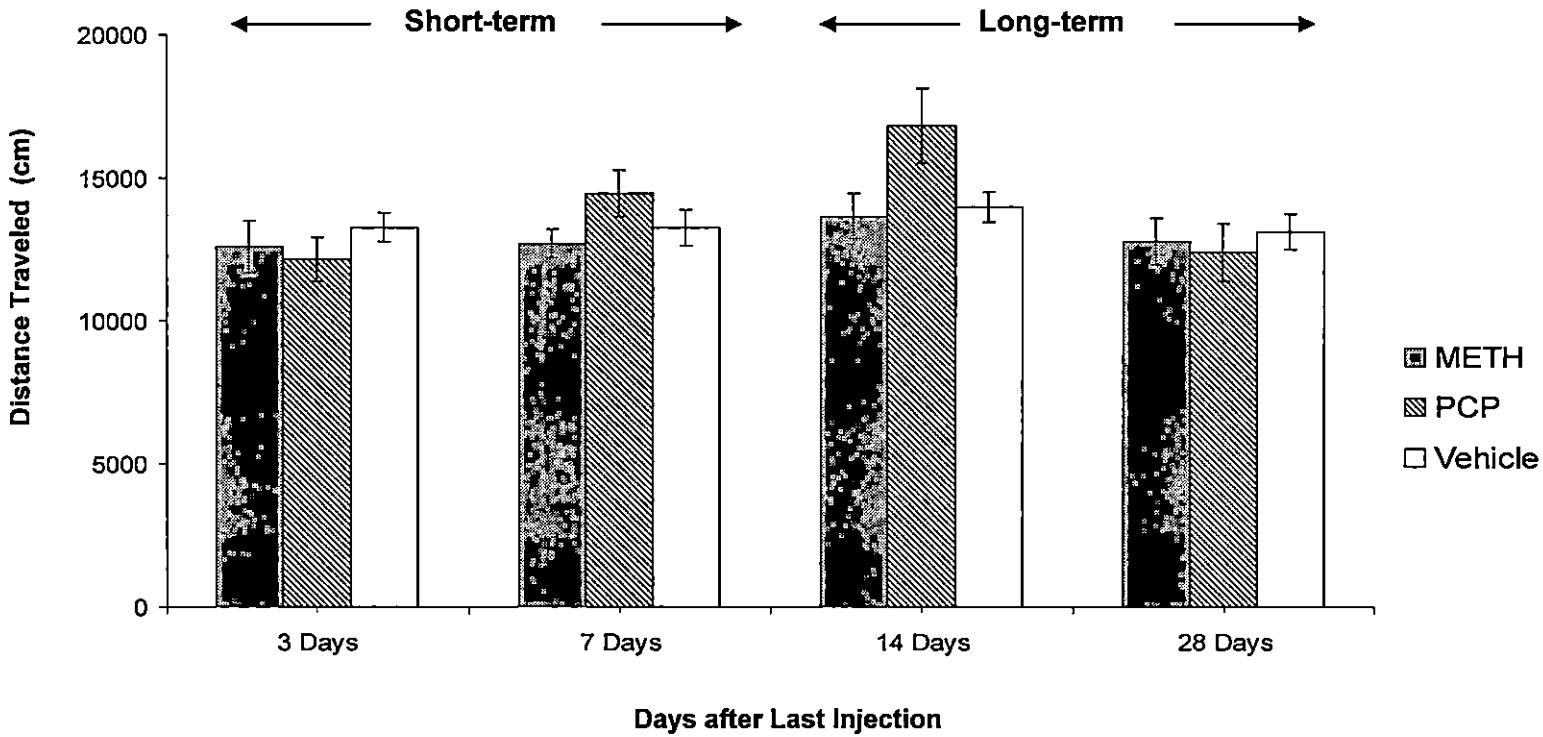


Figure 4. Effects of methamphetamine (METH) and phencyclidine (PCP) on total locomotion (60 min) during withdrawal (short- and long-term). The horizontal axis represents days after the last injection, and the vertical axis represents distance traveled during 60 min period. Error bars indicate SEM. METH and PCP did not affect overall locomotor activity during withdrawal periods.

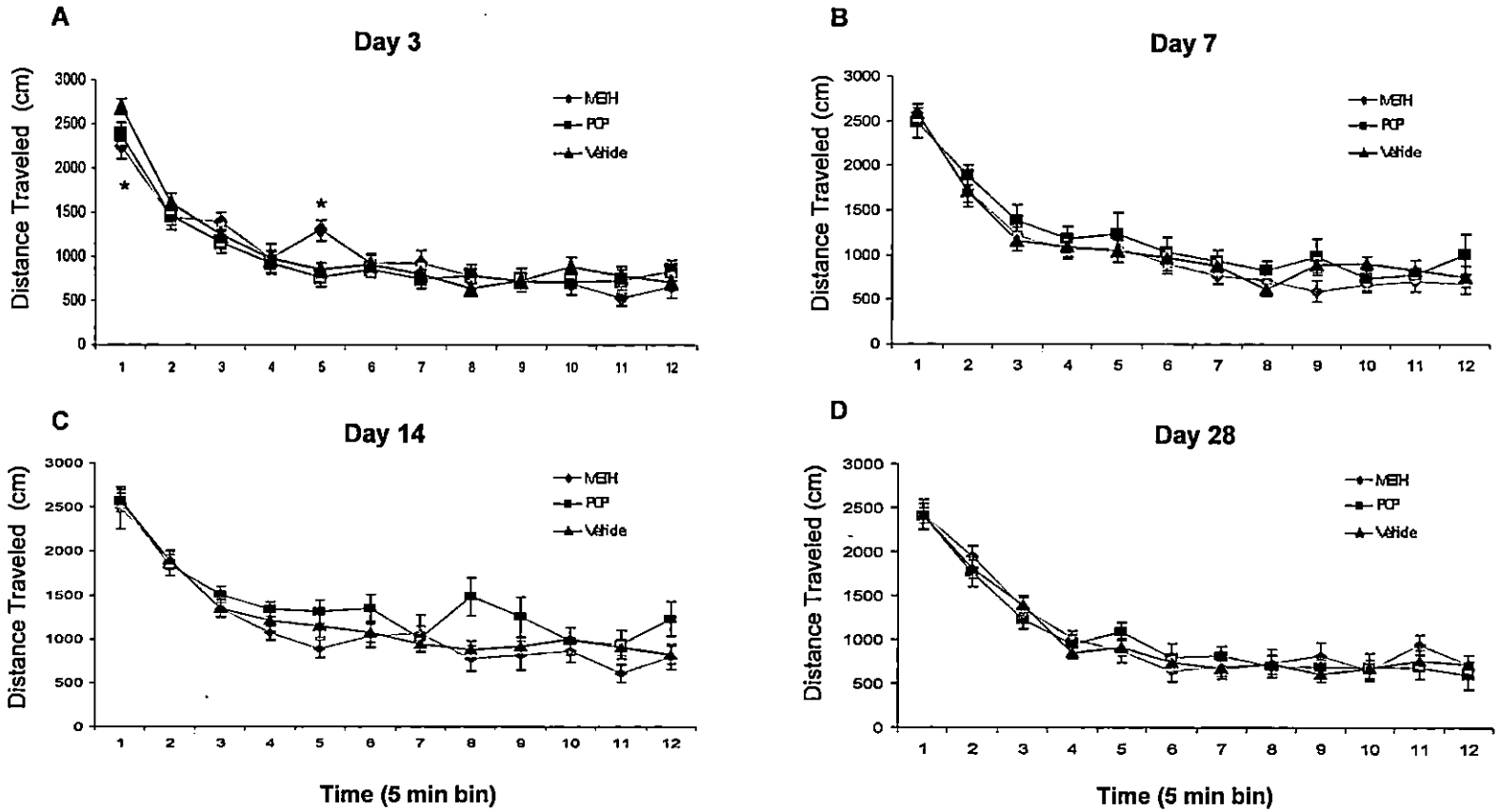


Figure 5. Effects of methamphetamine (METH) and phencyclidine (PCP) on locomotor activity during withdrawal. The horizontal axis represents the time (5 min bin) and the vertical axis represents distance traveled (cm). Error bars indicate SEM. On withdrawal Day 3 **(A)** METH produced hypolocomotion during the initial 5 min and hyperlocomotion between 20 min and 25 min, whereas PCP did not affect locomotor activity. On Days 7 **(B)**, 14 **(C)**, and 28 **(D)**, neither METH nor PCP affected locomotor activity. $*p < .05$.

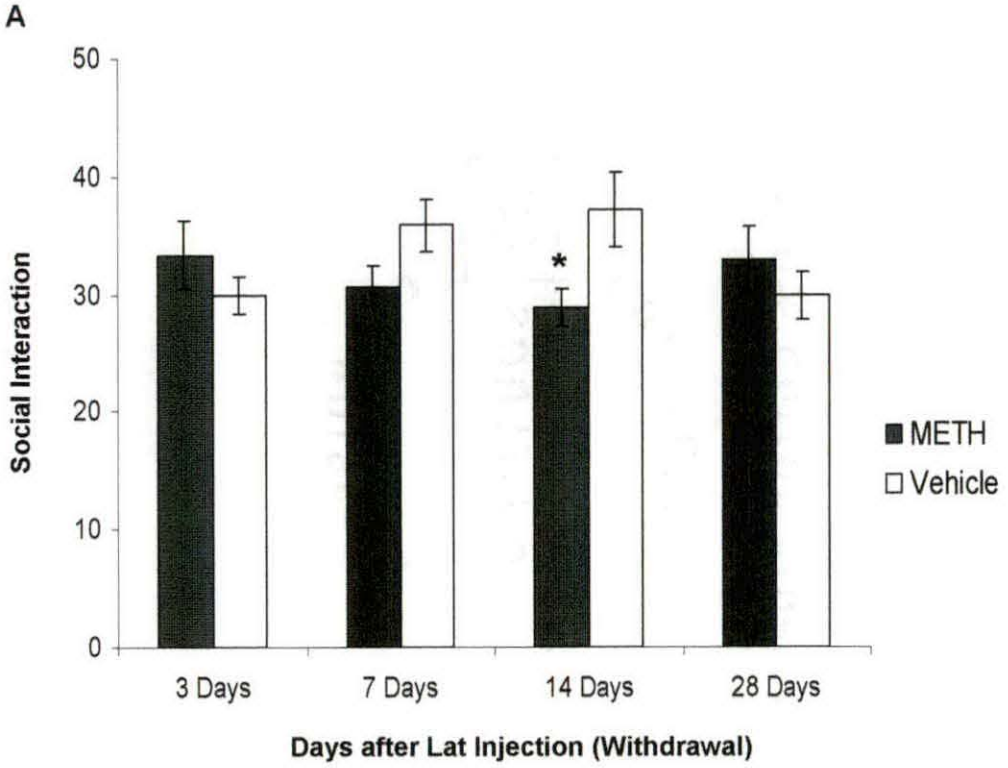
after the last injection, METH- and PCP-treated rats showed a locomotor activity comparable to that of saline-treated rats throughout the 60 min period (Fig. 5B, 5C, 5 D). A 3 x 12 ANOVA on the locomotion 3 days after the last injection yielded a significant interaction between treatment and time passage [F (22, 308) = 1.62, p < .05] and a significant effect of time passage [F (11, 308) = 67.09, p < .001], but did not show a significant treatment effect [F (2, 28) = 0.32, p > .05]. Post hoc analysis revealed significant differences between METH and saline treatment during the initial 5 min [t (21) = -2.68, p < .05], and between 25 and 30 min [t (21) = 3.31, p < .01]. A 3 x 12 ANOVA on the locomotion 7 days after the last injection showed a significant effect of time passage [F (11, 308) = 56.57, p < .001], but did not show a significant treatment effect [F (2, 28) = 1.24, p > .05] or interaction between treatment and time passage [F (22, 308) = 0.54, p > .05]. Likewise, a 3 x 12 ANOVA on the locomotion 14days after the last injection showed a significant effect of time passage [F (11, 308) = 51.42, p < .001], but did not show a significant treatment effect [F (2, 28) = 2.71, p > .05] or interaction between treatment and time passage [F (22, 308) = 1.17, p > .05]. A 3 x 12 ANOVA on the locomotion 28 days after the last injection yielded a significant effect of time passage [F (11, 308) = 83.46, p < .001], but did not show a significant treatment effect [F (2, 28) = 0.12, p > .05] or interaction between treatment and time passage [F (22, 308) = 0.59, p > .05].

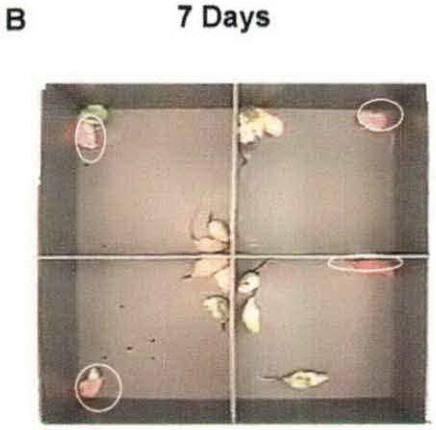
Experiment 2: Methamphetamine and PCP on Social Interaction: Short- and Long-term Withdrawal

Social interactions were measured during the withdrawal period: 3 days (PD 54), 7 days (PD 58), 14 days (PD 65) and 28 days (PD 79) after the last injection. Each drug treatment was compared with its paired vehicle, using 2 x 4 ANOVA to examine the main effect of the treatment and the main effect of days after the last injection. The withdrawal effects of PCP on initial 8 min of social interaction was also analyzed with a 2 x 4 ANOVA, consistent with previous studies, which measured 10 min social interaction (Sams-Dodd, 2004). Locomotor activity during the initial 8 min was also analyzed to examine the involvement of locomotor function on social interaction (2 x 4 ANOVA).

METH-treated rats showed a gradual decrease in social interaction 7 days and 14 days after the last injection, while METH- and saline-treated rats showed comparable social interaction 3 days and 28 days after the last injection (Fig. 6A) Compared to saline-treated rats, METH-treated rats showed a significantly decreased social interaction 14 days after the last injection. A 2 x 4 ANOVA yielded a significant interaction between treatment and days after the last injection [$F(3, 36) = 4.53, p < .01$], but did not show a significant treatment effect [$F(1, 12) = 0.59, p > .05$] or a significant effect of days after the last injection [$F(3, 36) = 0.50, p > .05$]. The following post-hoc analysis revealed a significant difference between METH and saline treatment 14 days after the last injection [$t(12) = -2.32, p = .038$].

PCP- and saline-treated rats showed comparable total social interaction (60 min) during the withdrawal period (Fig. 7A). Rats in both treatments showed decreased social interaction 14 days and 28 days after the last injection compared to





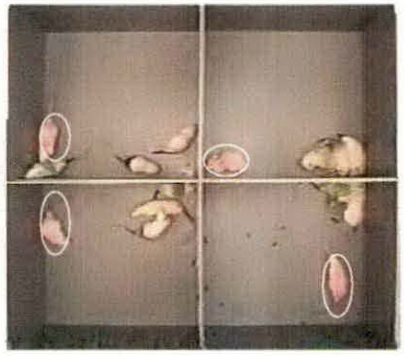


Figure 6. Effects of methamphetamine (METH) on social interaction during withdrawal. In figure **(A)**, the horizontal axis represents days after the last injection, and the vertical axis represents total score of social interaction during the 60 min period. Error bars indicate SEM. METH did not affect social interaction on Day 3. METH gradually decreased social interaction on Days 7 and 14. $*p < .05$. METH did not affect social interaction on Day 28. Pictures **(B)** and **(C)** show social interaction on Days 7 and 14 of withdrawal. Rats under each treatment were coded by color (red=METH; black=saline; green and yellow=no treatment).

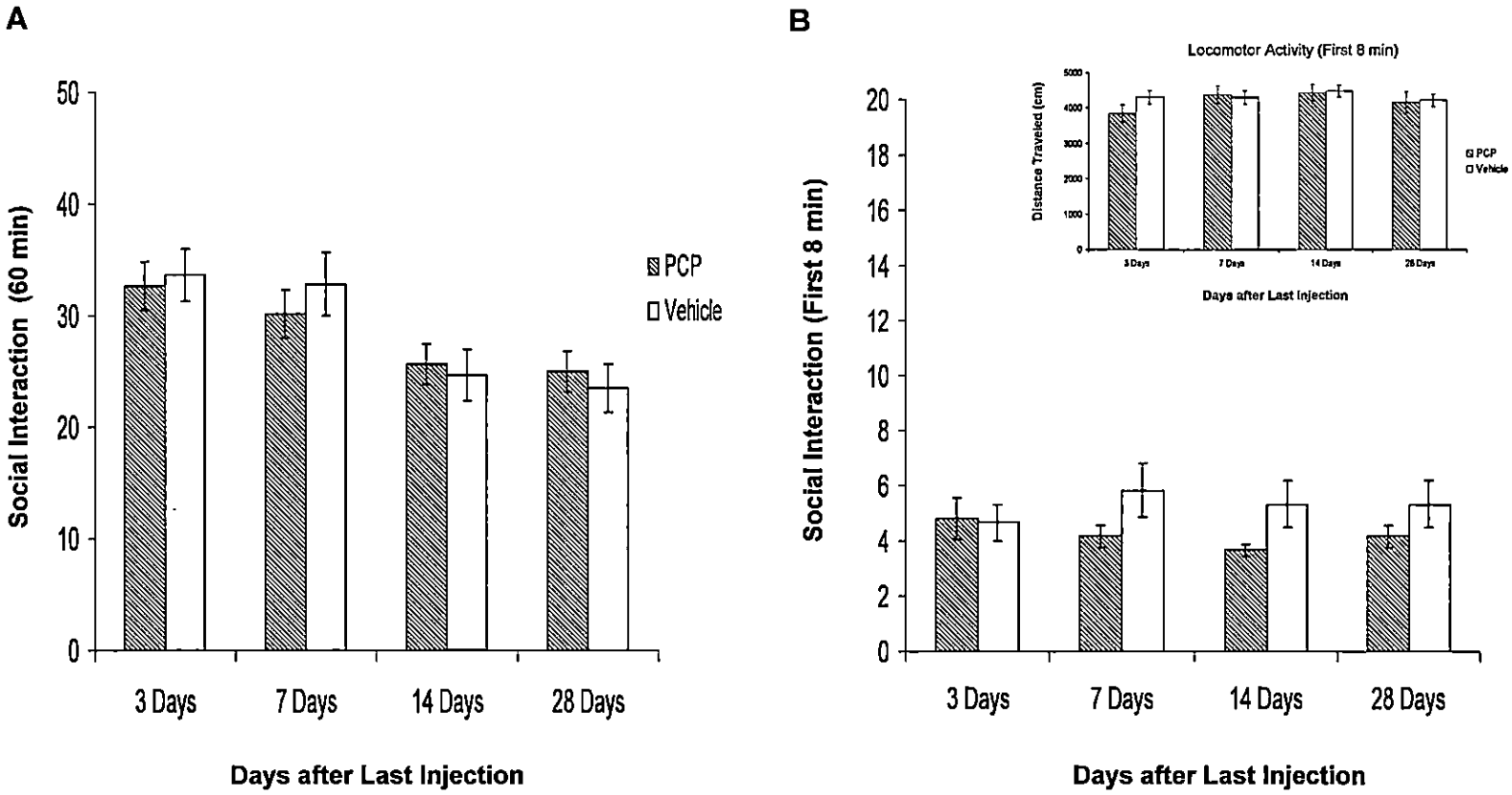


Figure 7. Effects of PCP on social interaction during withdrawal: **(A)** 60 min period, **(B)** initial 8 min period. PCP did not affect 60 min social interaction during the withdrawal periods **(A).** PCP-treated animals decreased social interaction during the first 8 min **(B).** Locomotor activity did not change during the withdrawal period **(inset)**. Error bars indicate SEM.

their social interaction 3 days and 7 days after the last injection. However, PCP-treated rats showed a decreasing trend in the initial 8 min of social interaction compared to saline-treated rats on Days 7, 14, and 28 of drug withdrawal (Fig. 7B). This decreasing trend in social interaction was not due to impairment of locomotor activity because PCP- and saline-treated rats showed a comparable 8 min locomotion during the withdrawal period. A 2 x 4 ANOVA on 60 min social interaction showed a significant effect of days after the last injection [F (3, 30) = 8.44, p < .001], but did not show a significant treatment effect [F (1 10) = 0.03, p > .05] or a significant interaction between treatment and days after the last injection [F (3, 30) = 0.39, p > .05]. Post hoc analysis revealed a significant difference in social interaction between Day 3 and Day 14 [t (12) = 3.06, p < .05], 3 days and 28 days [t (12) = 4.69, p < .001], 7 days and 14 days [t (12) = 2.56, p < .05] and 7 days and 28 days after the last injection [t (12) = 3.72, p < .01]. A 2 x 4 ANOVA on the initial 8 min showed a significant treatment effect [F (1, 10) = 5.65, p < .05], but did not show a significant effect of days [F (3, 30) = 0.17, p > .05] and a significant interaction between treatment and days after the last injection [F (1, 10) = 0.77, p > .05]. Pair-wise comparisons did not show a significant difference between PCP and saline treatment at any behavioral session, indicating that the significant treatment effect was due to the accumulated difference between PCP and saline treatment across sessions. A 2 x 4 ANOVA on 8 min locomotor activity showed a significant effect of days after the last injection [F (3, 63) = 2.93, p < .05]; however, the following post hoc analysis did not reveal any significant differences among the comparisons. ANOVA did not show a

significant treatment effect [F (1, 21) = 0.22, p > .05] or a significant interaction between treatment and days after the last injection [F (3, 63) = 1.42, p > .05].

Experiment 3: Methamphetamine and PCP on Spatial Discrimination and Reversal

To test the long-term effects of METH and PCP on spatial learning, acquisition of the spatial discrimination (SD) and the reversal task (SDR) were studied. Acquisition of SD, which required a barpresses opposite to the cue location, was assessed by percent correct responses, response latencies (correct and incorrect), and number of barpresses during the inter-trial intervals (ITI). In addition, number of days to reach the behavioral criterion (> 85 % correct response for 3 consecutive days) was measured. Once animals reached the behavioral criterion, acquisition of SDR, which required a barpresses same as the cue location, was assessed with the same test variables. Two-way and One-way ANOVA were used when appropriate. LSD pair-wise comparisons were performed for further analyses.

Effects of METH on Spatial Discrimination

In spatial discrimination, both saline- and METH-treated rats showed a gradual increase in the percent correct responses across training sessions (Fig. 8A). A 2 x 22 ANOVA yielded a significant effect of training session [F (21, 315) = 79.68, p < .001], but not a significant treatment effect [F (1, 15) = 0.31, p > .05] nor a significant interaction between treatment and training session [F (21, 315) = 0.60, p > .05].

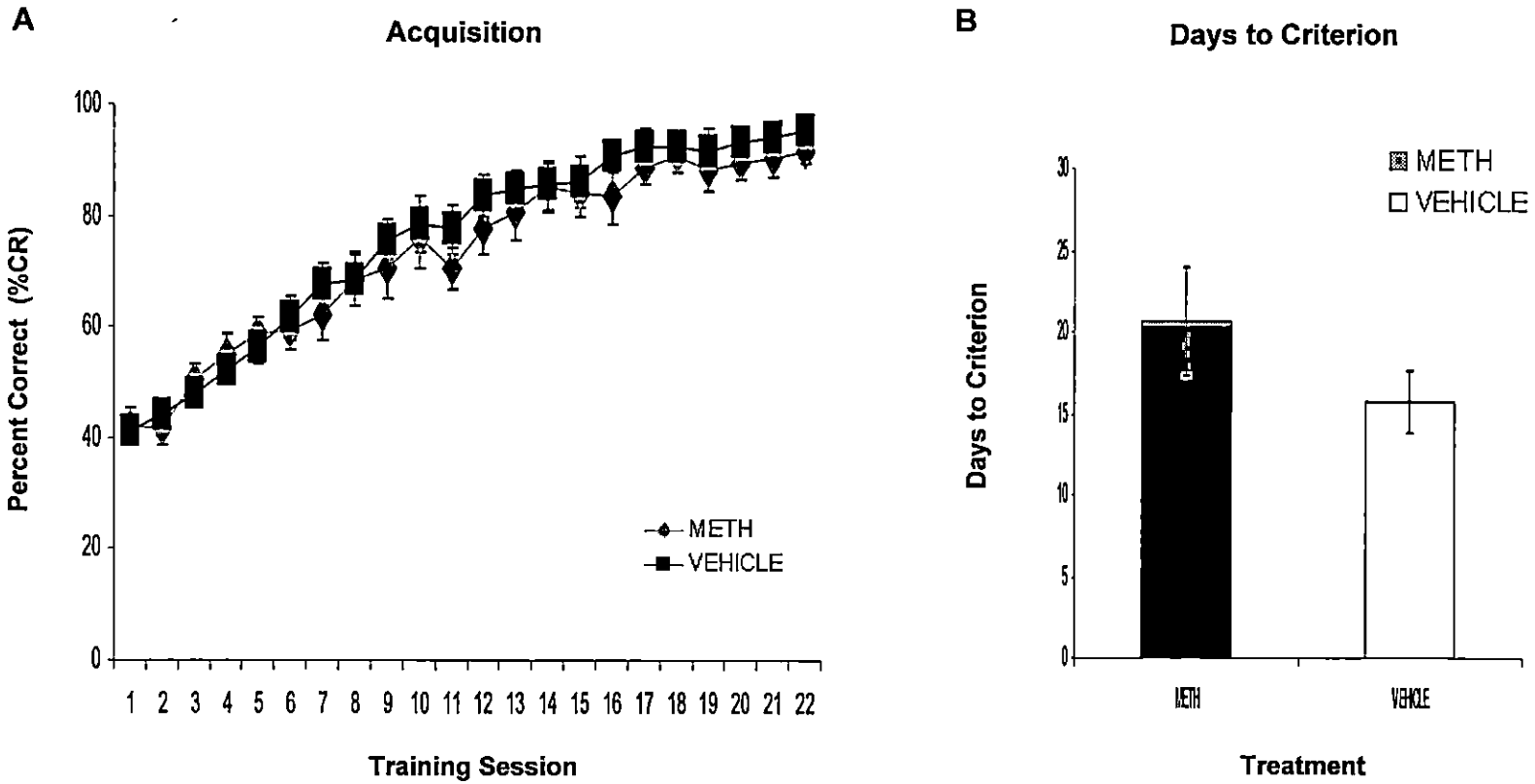


Figure 8. Effects of METH on spatial discrimination in adulthood. METH- and saline-treated rats showed a comparable increase in the percent correct responses across the training session **(A).** METH- and saline-treated rats required a similar number of training days to reach a criterion **(B).** Error bars indicate SEM.

METH- and saline-treated rats required a similar number of training days to reach the behavioral criterion (Fig. 8B). One-way ANOVA did not yield a significant treatment effect [$F(1, 15) = 1.79, p > .05$].

Correct response latency did not differ between METH- and saline-treated rats. Rats in both treatments showed relatively stable correct response latencies across the training sessions (Fig. 9A). A 2 x 22 ANOVA yielded a significant interaction between treatment and training session [$F(21, 315) = 1.87, p < .05$]. According to the post hoc analysis, the significant interaction was due to the slightly faster correct response latency in METH-treated rats on Days 15 and 19, however, no other major differences were observed. ANOVA did not show a significant treatment effect [$F(1, 15) = 1.13, p > .05$] or a significant effect of the training session [$F(21, 315) = 1.44, p > .05$].

Incorrect response latency did not differ between METH- and saline-treated rats. Saline-treated rats decreased incorrect response latencies across training sessions. Similarly, METH-treated rats showed a decrease in incorrect response latencies across training sessions (Fig. 9B). A 2 x 22 ANOVA yielded a significant effect of training session [$F(21, 315) = 12.94, p < .001$], but not a significant treatment effect [$F(1, 15) = 0.34, p > .05$] or a significant interaction between treatment and training session [$F(21, 315) = 0.46, p > .05$].

METH- and Saline-treated rats showed a gradual decrease in number of ITI barpresses during acquisition (Fig. 9C). A 2 x 22 ANOVA yielded a significant effect of training session [$F(21, 315) = 48.06, p < .001$], but not a significant treatment

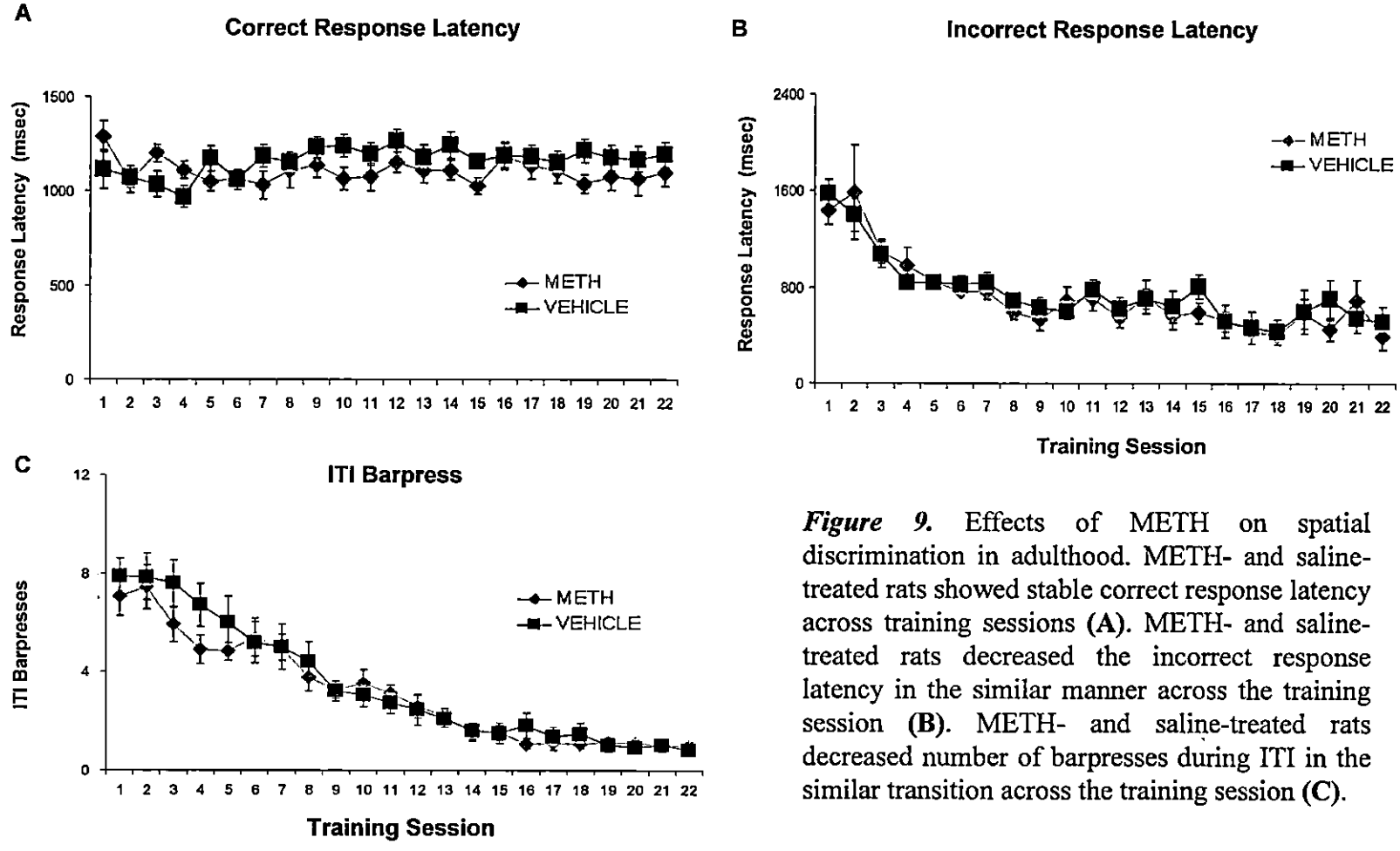


Figure 9. Effects of METH on spatial discrimination in adulthood. METH- and saline-treated rats showed stable correct response latency across training sessions **(A)**. METH- and saline-treated rats decreased the incorrect response latency in the similar manner across the training session **(B)**. METH- and saline-treated rats decreased number of barpresses during ITI in the similar transition across the training session **(C)**.

effect [F (1, 15) = 0.36, p > .05] or a significant interaction between treatment and training session [F (21, 315) = 0.92, p > .05].

Thus, METH- and saline-treated rats required a comparable number of training days to acquire spatial discrimination and had similar pattern of percent correct responses, response latencies, and number of barpress during the ITI across the training sessions.

Effects of METH on Reversal

In the reversal task saline-treated rats gradually increased percent correct responses as the training sessions progressed. METH-treated rats showed a slightly slower increase in percent correct responses compared to saline-treated rats (Fig. 10A). A 2 x 22 ANOVA showed a trend toward a treatment effect [F (1, 15) = 3.30, p = 0.08] and a significant effect of training session [F (21, 315) = 54.17, p < .001]. The interaction between treatment and training session was not significant [F (21, 315) = 0.61, p > .05]. METH- and saline-treated rats required a similar number of training days to reach the behavioral criterion (Fig. 10B). One-way ANOVA did not yield a significant treatment effect [F (1, 15) = 2.11, p > .05]. Although there were no significant treatment effects in percent correct responses and days to criterion, METH-treated rats showed a trend toward a slower increase in percent correct responses. Post hoc pair-wise comparisons revealed significant differences between METH and saline treatment on training session 6, 12, 18, 20, 21, and 22 ($p < 0.05$), indicating that METH-treated rats showed a poor performance during reversal toward the end of the

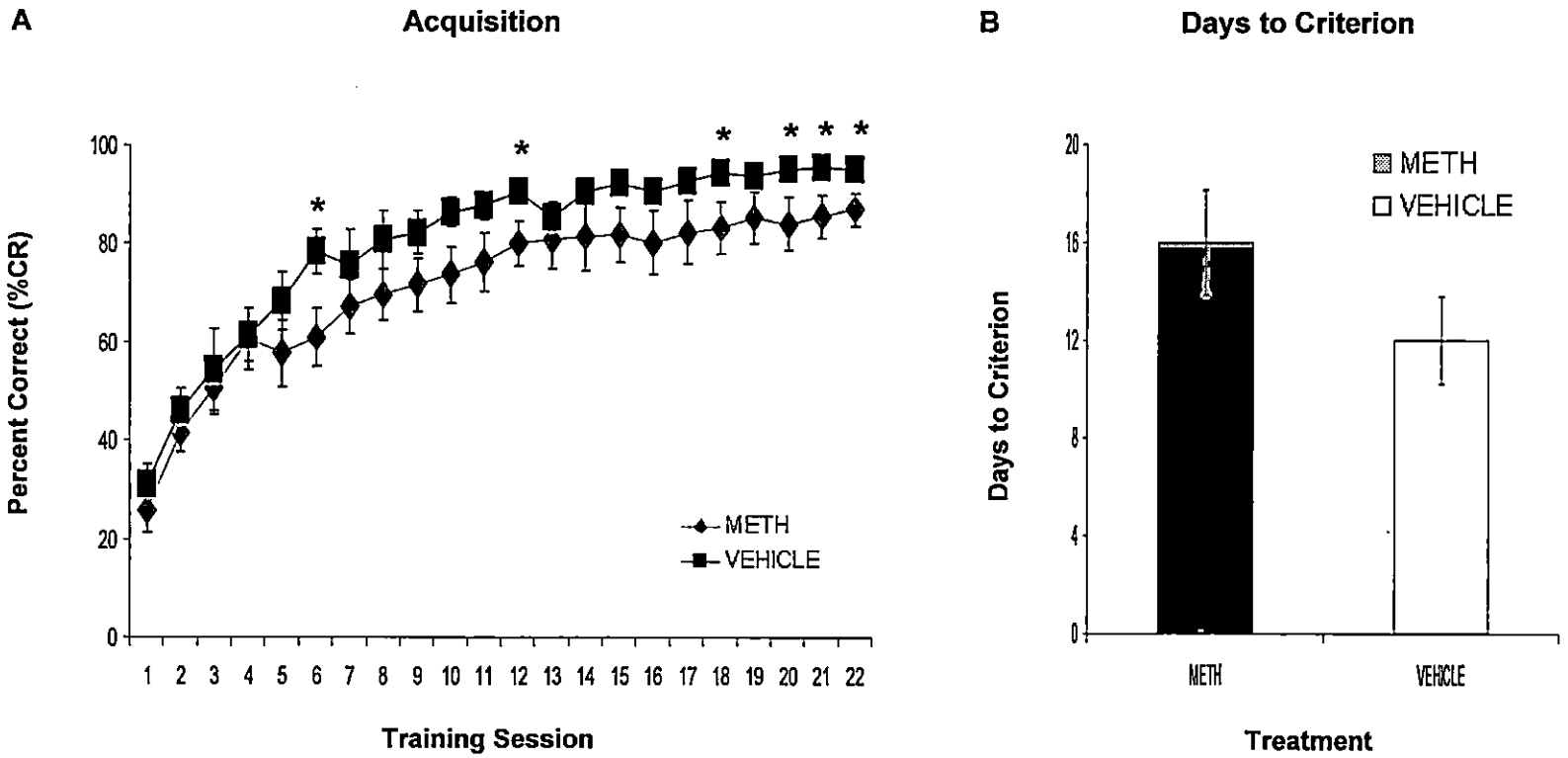


Figure 10. Effects of METH on reversal learning in adulthood. METH-treated rats showed a slower trend in increasing the percent correct responses compared with saline-treated rats **(A)**. METH- and saline-treated rats required similar number of training days to reach the behavioral criterion **(B)**. $*p < .05$.

training session.

Correct response latency did not differ between METH- and saline-treated rats. Saline-treated rats showed stable correct response latency across all the training sessions. Similarly, METH-treated rats showed consistent correct response latencies across all the training sessions (Fig. 11A). Although a 2 x 22 ANOVA yielded a significant effect of training session [F (21, 315) = 2.03, p < .01], the following post hoc analysis did not show any difference among the possible comparisons. There was no significant treatment effect [F (1, 15) = 0.01, p > .05] or a significant interaction between treatment and training session [F (21, 315) = 0.56, p > .05].

Incorrect response latency did not differ between METH- and saline-treated rats. Saline-treated rats gradually decreased incorrect response latency across the training sessions. Similarly, METH-treated rats showed a gradual decrease in incorrect response latency across the training session (Fig. 11B). A 2 x 22 ANOVA yielded a significant effect of training session [F (21, 315) = 8.17, p < .001] but did not show a significant treatment effect [F (1, 15) = 0.05, p > .05] or a significant interaction between treatment and training session [F (21, 315) = 0.80 p > .05].

Saline-treated rats gradually decreased the number of barpresses during the ITI across the training sessions. Similarly, METH-treated rats showed a gradual decrease in number of barpresses during the ITI across the training session (Fig. 11C). A 2 x 22 ANOVA showed a significant effect of training session [F (21, 315) = 9.60, p < .001], but did not show a significant treatment effect [F (1, 15) = 0.49, p > .05] or a significant interaction between treatment and training session [F (21, 315) = 0.32, p

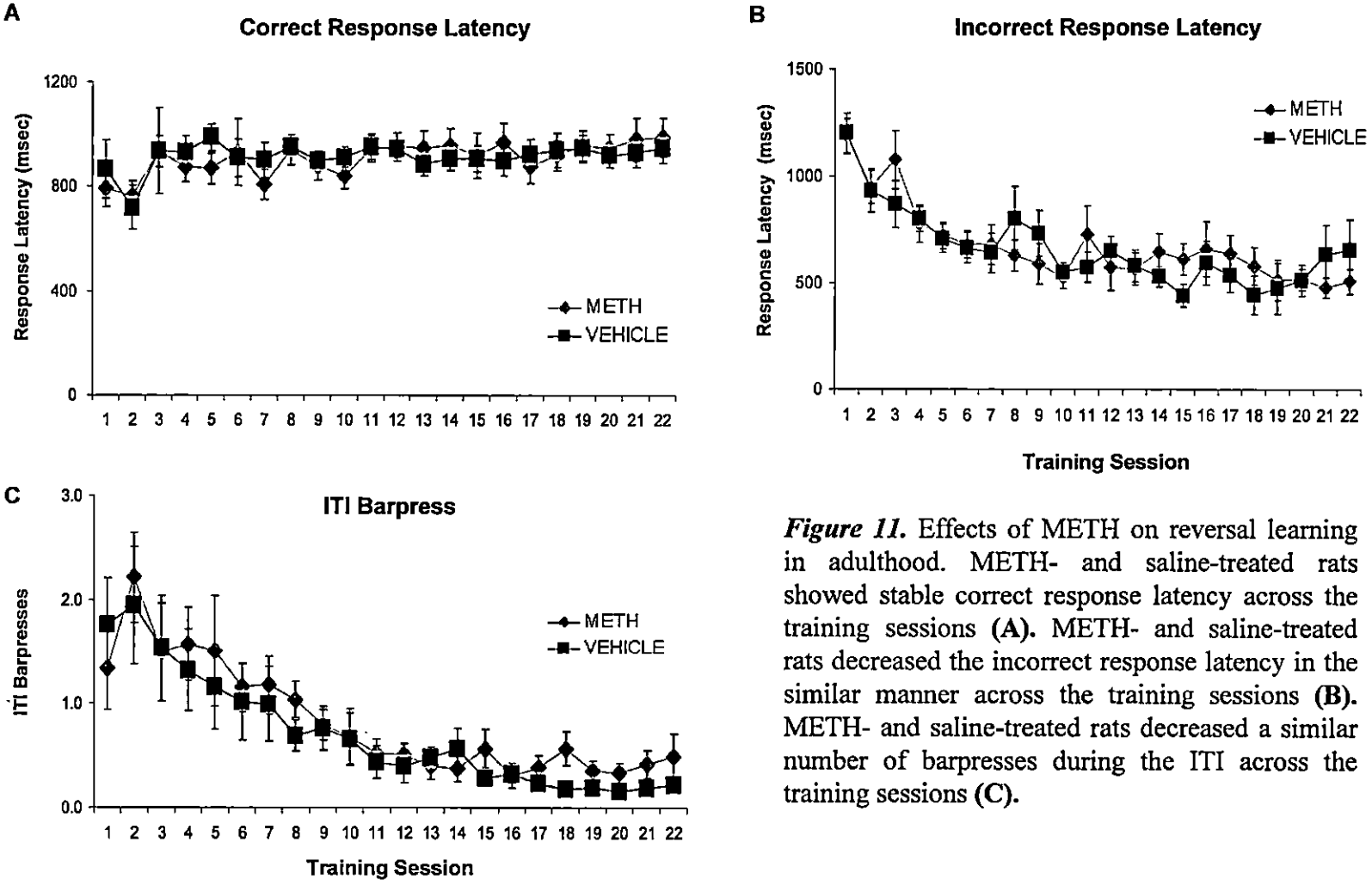


Figure 11. Effects of METH on reversal learning in adulthood. METH- and saline-treated rats showed stable correct response latency across the training sessions **(A).** METH- and saline-treated rats decreased the incorrect response latency in the similar manner across the training sessions **(B).** METH- and saline-treated rats decreased a similar number of barpresses during the ITI across the training sessions **(C).**

> .05].

Thus, METH- and saline-treated rats took a comparable number of training days to acquire SDR and were similar in terms of response latencies and the number of barpresses during the ITI across the training sessions. However, during reversal METH-treated rats showed a trend toward a slower increase in percent correct responses toward the end of the training session.

Effects of PCP on Spatial Discrimination

In the spatial discrimination task saline-treated rats gradually increased percent correct responses as training progressed. Similarly, PCP-treated rats showed a gradual increase in the percent correct responses with training (Fig. 12A). A 2 x 22 ANOVA yielded a significant effect of the training session [$F(21, 315) = 113.74, p < .001$], but did not show a significant treatment effect [$F(1, 15) = 1.44, p > .05$] or a significant interaction between treatment and training session [$F(21, 315) = 1.08, p > .05$]. PCP- and saline-treated rats required a similar number of training days to reach the behavioral criterion (Fig. 12B). One-way ANOVA did not yield a significant treatment effect [$F(1, 15) = 1.31, p > .05$].

Correct response latency did not differ between PCP- and saline-treated rats. Saline-treated rats showed stable correct response latencies across the training session. Similarly, PCP-treated rats showed consistent correct response latencies across the training session (Fig 13A). Although a 2 x 22 ANOVA yielded a significant effect of the training sessions [$F(21, 315) = 2.35, p < .01$], this was due to the slightly faster

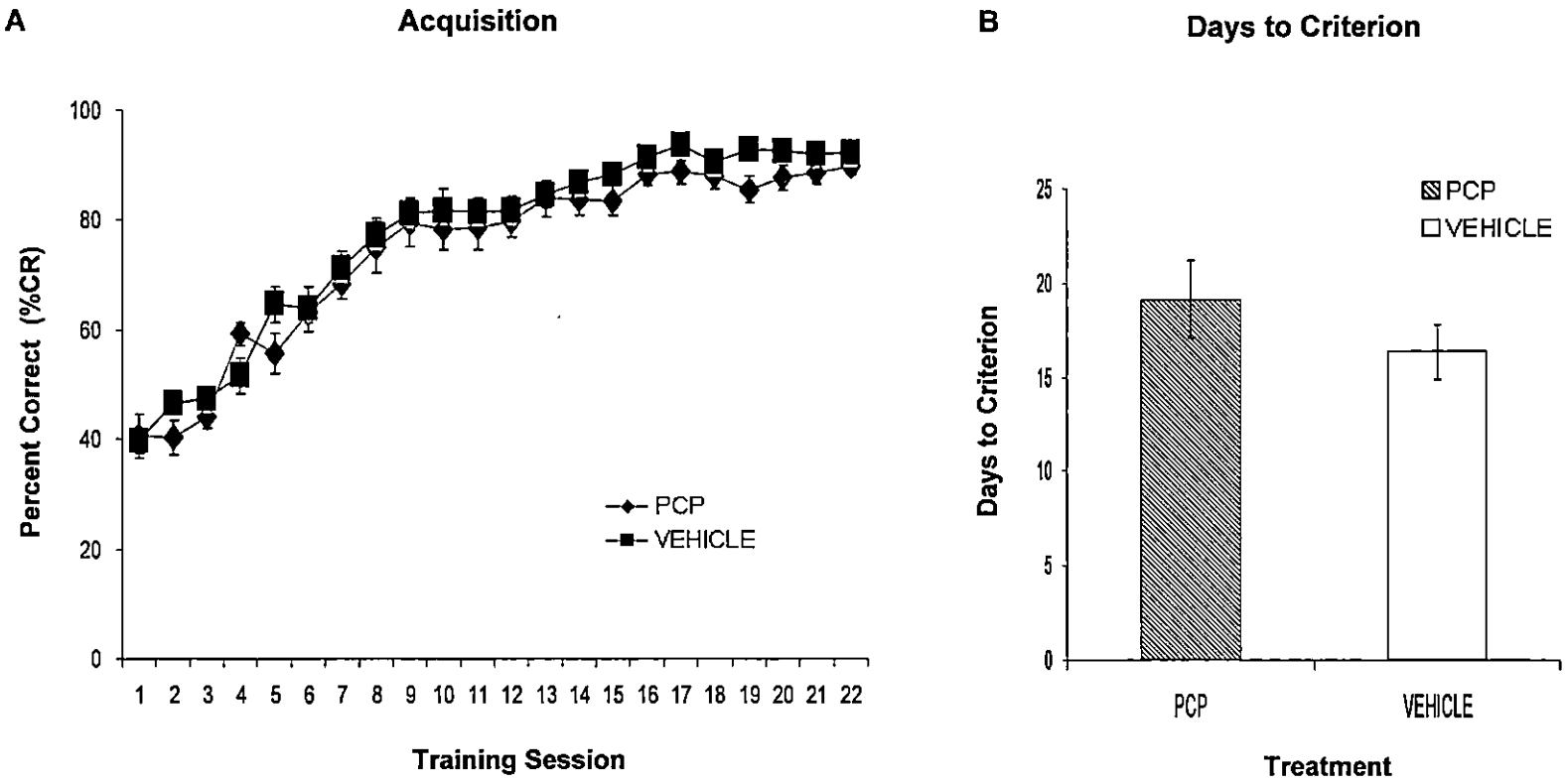


Figure 12. Effects of PCP on spatial discrimination in adulthood. PCP- and saline-treated rats showed a comparable increase in the percent correct responses across the training sessions **(A).** PCP- and saline-treated rats required similar number of training days to reach the behavioral criterion **(B).**

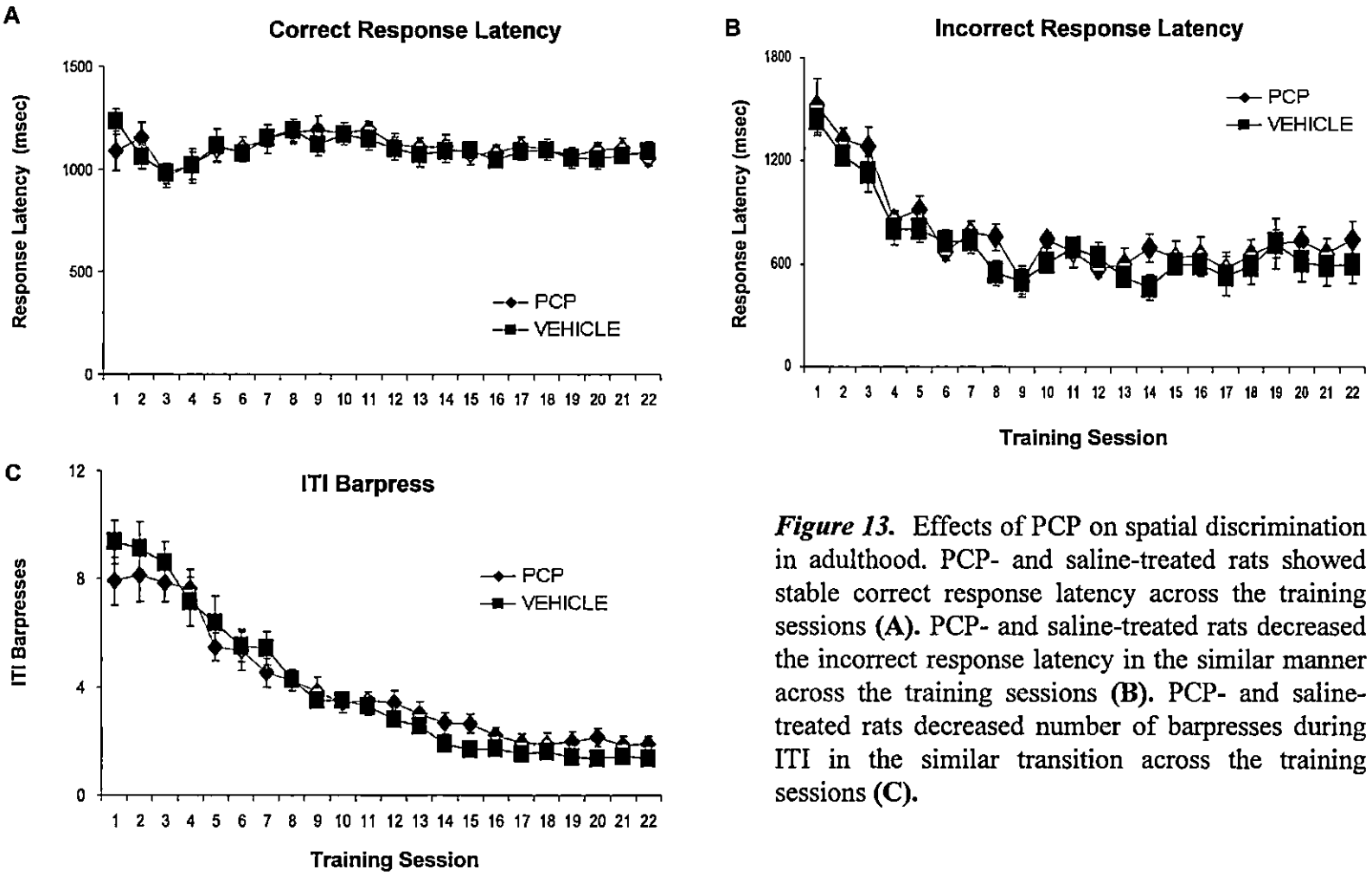


Figure 13. Effects of PCP on spatial discrimination in adulthood. PCP- and saline-treated rats showed stable correct response latency across the training sessions **(A)**. PCP- and saline-treated rats decreased the incorrect response latency in the similar manner across the training sessions **(B)**. PCP- and saline-treated rats decreased number of barpresses during ITI in the similar transition across the training sessions **(C)**.

correct response latency on Day 3 of the training session, and no other significant differences were obtained in the post hoc analysis. There were no significant treatment effect [$F(1, 15) = 0.08, p > .05$] or a significant interaction between treatment and training session [$F(21, 315) = 0.48, p > .05$].

Incorrect response latency did not differ between PCP- and saline-treated rats. Saline-treated rats decreased incorrect response latencies across the training sessions. Similarly, PCP-treated rats showed a decrease in incorrect response latencies across the training sessions (Fig. 13B). A 2 x 22 ANOVA yielded a significant effect of training session [$F(21, 315) = 21.31, p < .001$], but did not show a significant treatment effect [$F(1, 15) = 1.51, p > .05$], or a significant interaction between treatment and training session [$F(21, 315) = 0.48, p > .05$].

Saline-treated rats gradually decreased the number of barpresses during the ITI across the training sessions. Similarly, PCP-treated rats showed a gradual decrease in number of barpresses during the ITI (Fig 13C). A 2 x 22 ANOVA yielded a significant effect of training session [$F(21, 315) = 69.01, p < .001$], but did not show a significant treatment effect [$F(1, 15) = 0.05, p > .05$] or a significant interaction between treatment and training session [$F(21, 315) = 1.33, p > .05$].

Thus, PCP- and saline-treated rats required comparable number of training days to acquire SD with similar transitions in the percent correct responses, response latencies, and number of barpress during ITI across the training session.

Effects of PCP on Reversal

Saline-treated rats increased percent correct responses during the earlier training

sessions, and remained at asymptote during the remaining sessions. On the other hand, PCP-treated rats showed a slower increase in the percent correct responses compared to saline-treated rats (Fig. 14A). A 2 x 22 ANOVA yielded a significant treatment effect [$F(1, 15) = 4.54, p < .05$], and the following post hoc analysis revealed a significant difference between PCP and saline treatment on Days 6, 10, 12, 15, 18 and 20 of the training sessions. ANOVA also showed a significant effect of the training session [$F(21, 315) = 117.94, p < .001$] but not a significant interaction between treatment and training session [$F(21, 315) = 0.69, p > .05$]. Compared to saline-treated rats, PCP-treated rats required more training days to reach the behavioral criterion (Fig. 14B). One-way ANOVA showed a significant treatment effect on days to reach the behavioral criterion [$F(1, 15) = 6.53, p < .05$].

Correct response latency did not differ between PCP- and saline-treated rats. Saline-treated rats showed stable correct response latency across the training session. Similarly, PCP-treated rats showed constant correct response latency across the training session (Fig. 15A). A 2 x 22 ANOVA did not yield a significant treatment effect [$F(1, 15) = 1.67, p > .05$], a significant effect of training session [$F(21, 315) = 1.22, p > .05$] and a significant interaction between treatment and training session [$F(21, 315) = 1.38, p > .05$].

Incorrect response latency did not differ between PCP- and saline-treated rats. Saline-treated rats decreased incorrect response latency across the training session. Similarly, PCP-treated rats showed a decrease in the incorrect response latency across the training session (Fig. 15B). A 2 x 22 ANOVA yielded a significant

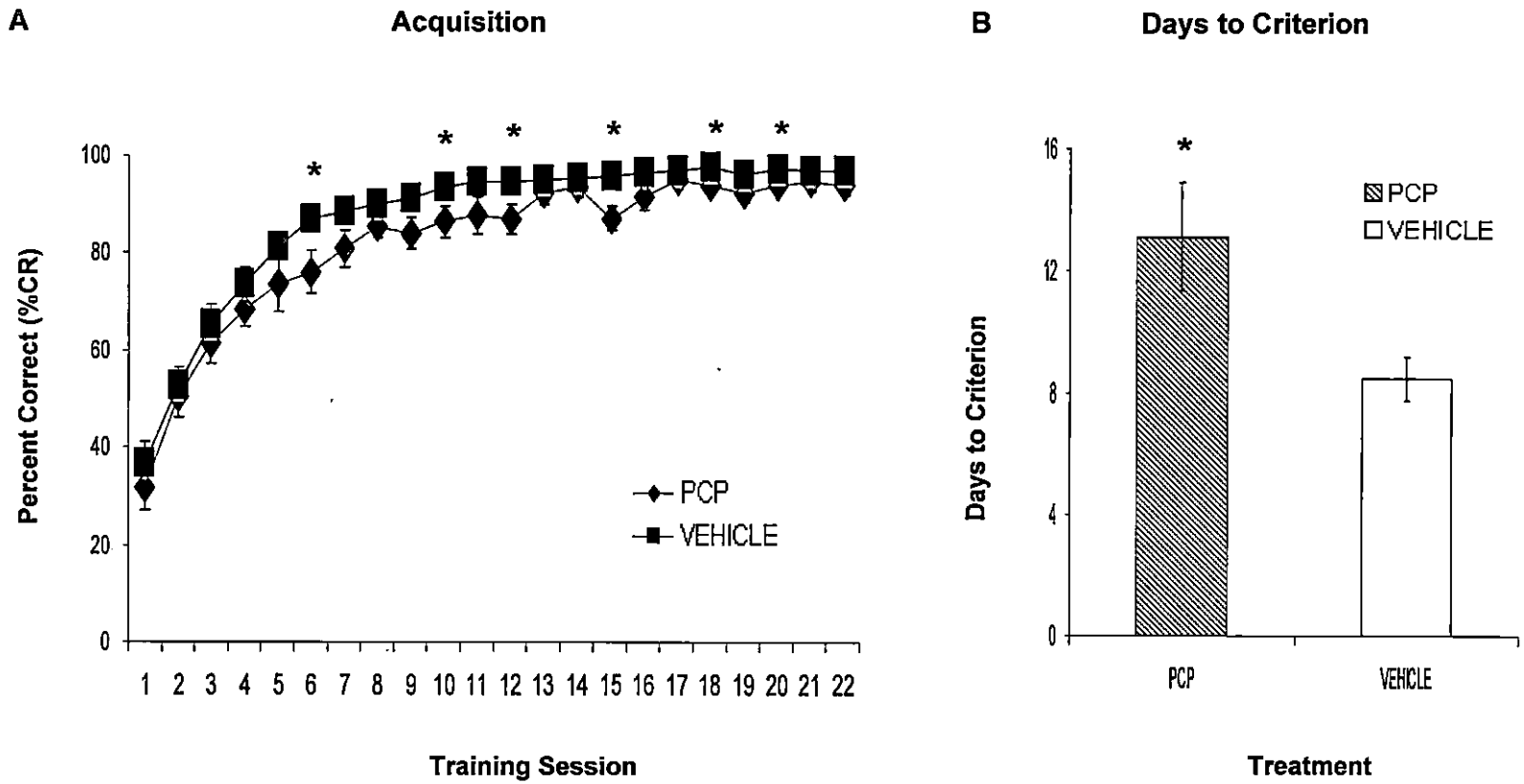


Figure 14. Effects of PCP on reversal learning in adulthood. PCP-treated rats showed a slower increase in the percent correct responses in comparison with saline-treated rats **(A).** PCP-treated rats required longer training days to reach the behavioral criterion compared with saline-treated rats **(B).** $*p < .05$.

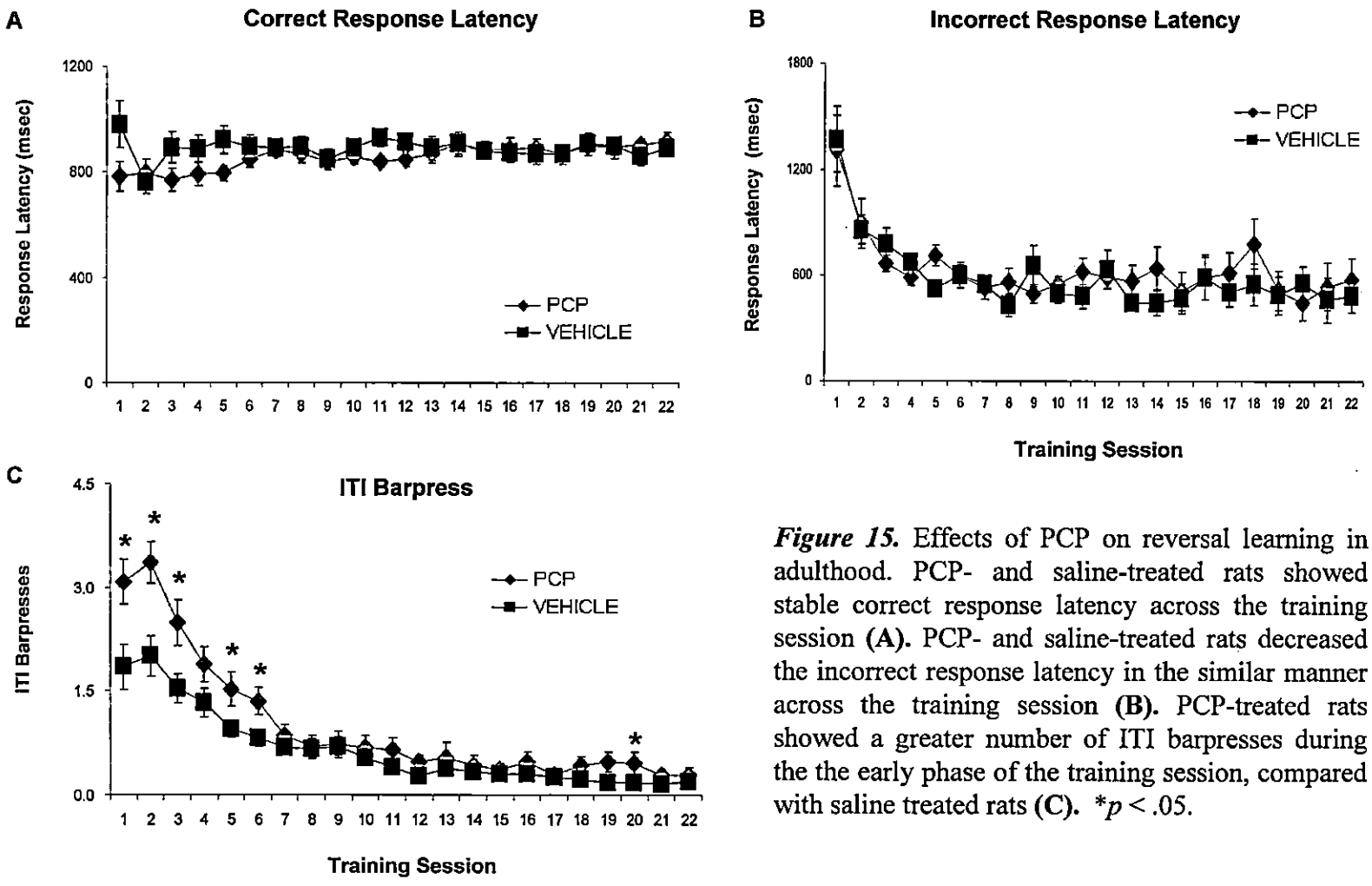


Figure 15. Effects of PCP on reversal learning in adulthood. PCP- and saline-treated rats showed stable correct response latency across the training session **(A).** PCP- and saline-treated rats decreased the incorrect response latency in the similar manner across the training session **(B).** PCP-treated rats showed a greater number of ITI barpresses during the the early phase of the training session, compared with saline treated rats **(C).** $*p < .05$.

effect of the training session [F (21, 315) = 7.82, p < .001], but did not show a significant treatment effect [F (1, 15) = 0.57, p > .05] and a significant interaction between treatment and training session [F (21, 315) = 0.66, p > .05].

Saline-treated rats decreased number of barpresses during ITI across the training session. Although PCP-treated rats also showed a decrease in number of barpresses across the training session, these animals showed a greater number of bar-press during the earlier phase of the training session compared to saline-treated animals (Fig. 15C). A 2 x 22 ANOVA yielded a significant treatment effect [F (1, 15) = 5.93, p < .05], a significant effect of the training session [F (21, 315) = 58.54, p < .001] and a significant interaction between treatment and training session [F (21, 315) = 3.89, p < .001]. Post hoc analysis revealed a significant difference between PCP and saline treatment on Days 1, 2, 3, 5, 6, and 19 of the training sessions.

Thus, compared to saline-treated rats, PCP-treated rats required a greater number of days to acquire SDR and had a slower increase in the percent correct responses. In addition, PCP-treated rats showed a greater number of barpresses during the ITI in the earlier training phase in comparison with saline-treated animals. PCP- and saline-treated rats showed comparable changes in response latencies across the training sessions.

Discussion

Effects of Methamphetamine and Phencyclidine on Locomotor Activity

Acute Drug State. METH and PCP differentially affected locomotor activity after the first and the third injections. Following the first METH injection locomotor activity was markedly enhanced during the first half of the session, whereas the third injection did not affect locomotion. On the other hand, the first PCP injection enhanced locomotion during the second half of the session, whereas the third PCP injection enhanced locomotion throughout the entire session. Acute effects of METH and PCP are further discussed below.

Enhanced locomotion (hyperlocomotion) following the first METH injection peaked during the first 10 min after the injection and lasted for 25 min. This initial behavioral excitation may reflect changes in dopamine transmission in the nucleus accumbens, which is thought to regulate locomotor activity (Tran et al., 2004). Thus, decreased locomotor activity during the second half of the session may reflect some change of DA in the nucleus accumbens. Shoblock et al. (2003) found that METH exponentially increased DA in the NAc 20 - 40 min after the injection (2 mg/kg, i.p.). The time course of DA increase in the NAc seen in Shoblock et al's study is similar to a decrease in locomotion seen in the present study. This is likely due to dose differences: 9 mg/kg (present study) vs. 2mg/kg (Shoblock et al, 2003). Interestingly, the third METH injection did not affect locomotor activity. This lack of locomotion is probably due to the cumulative METH in the system from the second injection, which in turn promoted further DA increase in the nucleus accumbens following the third injection. In fact, Brooks et al. (2004) showed that a single injection of a moderate dose (3 mg/kg) of METH produced hyperlocomotion, which lasted 300 min (5 hrs),

while lower doses (0.3 - 1 mg/kg) produced hyperlocomotion for 100-200 min, respectively. In the present study a relatively high dose (9 mg/kg) of METH was injected at 12 hr intervals. Thus, the residual effects from previous injections in addition to the third injection produced excessive DA in the nucleus accumbens, leading to a decrease in locomotion. Moreover, this biphasic pattern of locomotor activity in the present data is consistent with previous reports that a single injection of low vs. high doses of METH produced different locomotor patterns. For example, a single injection of low doses (1-2 mg/kg) of METH produced continuous hyperlocomotion (Mori et al., 2004; Shoblock et al., 2003), whereas moderate to high doses of METH (3-20 mg/kg) enhanced locomotion briefly, followed by a decrease in locomotion (Brooks et al., 2004; Mori et al., 2004; Shoblock et al., 2003). Thus, the present data suggest that the pattern of locomotor activity depends on the dose and frequency of METH injection. Given that METH increases DA in the NAc (Shoblock et al., 2003), the present findings also indicate that although enhanced dopamine in the nucleus accumbens is required to produce hyperlocomotion, changes in locomotor activity are sensitive to a moderate, but not an excessive increase in DA.

PCP produced opposite effects to that of METH on locomotor activity. The first PCP injection did not affect locomotion during the first half of the session. However, PCP increased locomotion during the second half of the session. The delayed onset of increase in locomotion may be due to the indirect effect of PCP on DA transmission in the NAc. PCP is known to block the PCP-site of NMDA receptors (Zukin & Javitt, 1993) and is implicated in an increase in extracellular DA

concentration in the NAc (Hanson et al., 1995), indicating the indirect action of PCP. In fact, Nishijima et al. (1996) reported that PCP increased DA level in the striatum 20-40 min after the PCP injection (2.5-10 mg/kg, i.p.). The time course of DA increase in the striatum in Nishijima et al's study corresponds to the onset of hyperlocomotion in the present study. Thus, the delayed onset of hyperlocomotion may reflect the indirect action of PCP on the DA increase in the NAc. Locomotor excitation during the second half of the session may have been due to the moderate increase of DA in the NAc. Unlike METH, which produces prolonged hyperlocomotion at only at low doses (Shoblock et al. 2003), PCP (2.5 – 10mg/kg) enhances locomotion in a dose-dependent manner (Tani et al, 1994). Moreover, METH (4.8 mg/kg) augmented peak DA in the NAc nearly fivefold compared to PCP (10 mg/kg) (Shimada, et al. 1996). These data indicate that PCP moderately increases DA in the NAc and produces hyperlocomotion. Interestingly, the third PCP injection further increased locomotor activity throughout the session. One explanation for the further increase in locomotion after the third PCP injection is that a carryover effect from the previous two injections combined with the third injection, and further increased DA level in the NAc. Nevertheless, hyperlocomotion continued to rise throughout the session, indicating an indirect DA increase in the NAc. It is also possible that locomotor activity was sensitized by the previous two injections. Locomotor sensitization produced by the third PCP injection (3.2 mg/kg at 24 hr interval) was also reported in a previous study (Xu & Domino, 1993).

Taken together, although METH and PCP affected locomotor activity, each

drug produced distinctively different effects on locomotion. The first METH injection markedly increased locomotor activity during the first half of the session, whereas the third injection did not affect locomotion. On the other hand, the first PCP injection produced hyperlocomotion during the second half of the session, whereas the third injection enhanced locomotion throughout the entire session. This difference is probably due to the different action of METH and PCP on the NAc. METH directly affects the NAc and increases DA excessively, whereas PCP indirectly affects the NAc and increases DA moderately. The acute effects of METH and PCP on locomotor activity in the present study suggest that locomotor activity is sensitive to a moderate increase of DA in the NAc but not an excessive increase.

Withdrawal State (Short- and Long-term). With an exception of withdrawal Day 3, both METH and PCP produced similar effects on locomotor activity during short- and long-term withdrawal periods, which spanned Days 3- 28 from the last drug injections.

Three days after the last METH injection, the METH group showed slightly suppressed locomotion during the first 5 min only, compared to the control. Since rats tend to get engaged in exploratory behavior at the beginning of the testing period, a decrease in distance traveled during the first 5 min period may reflect a locomotor deficit. Timar et al. (2003) measured locomotor activity in a novel environment and found a decrease in locomotion 3 days after METH (10 mg/kg, s.c. x 4 injections at a 2 hr interval). Although in Timar et al's study locomotor activity was tested without a habituation session (i.e. novel environment), their results are in agreement with the

present study. In the present study, well-habituated rats also showed a decrease in locomotion 3 days after injections. It is possible that this hypolocomotion during the first 5 min reflects DA depletion in the NAc. In fact, METH (5 mg/kg, s.c. x 5 daily injection) increased the sensitivity of neurons in the NAc 5 days after the administration (Amano, et al. 1996). Locomotor activity was not affected 7-28 days post injections of METH in the present study. Previous study also reported normalized locomotor activity at the same testing period (Timar et al. 2003). Moreover, Amano et al. (1996) reported normal neuronal activity in the NAc 10 days post administration of METH. This normalized locomotion 7-28 days post injections may reflect the normal level of DA in the NAc.

PCP produced no effects on locomotor activity during acute- or long-term withdrawal periods. While the present data indicate that PCP affects locomotion only during the acute state, previous reports indicated that repeated treatment with PCP produced a prolonged hypersensitivity. For example, following PCP treatment (20 mg/kg/day x 5 days), a challenge dose of PCP (3.2 mg/kg, i.p.) produced sensitization during withdrawal periods 3 and 8 days after the PCP injection (Hanania et al., 1999). The authors suggested that repeated PCP treatment produced a prolonged hypersensitivity, possibly due to altered neurotransmitter systems (Hanania et al., 1999). These findings are inconsistent with findings in the present study. One explanation is that the dose of PCP used in Hanania et al's study is nearly three-folds higher than the dose used in the present study, thereby producing a greater degree of neurotoxicity. According to Bella et al. (2003), sensitivity of DA receptors in the

prefrontal cortex was restored 4 days after withdrawal from PCP treatment (15 mg/kg/day for 2 weeks). Though Bella et al's study investigated the withdrawal effect only 4 days after the treatment, it is highly likely that DA level in the prefrontal cortex was recovered 3 days after the treatment, the time at which locomotor activity was tested in the present study.

Taken together, the present data indicate that locomotor activity is severely affected immediately after METH (9.0 mg/kg x 4 times) or PCP (9.0 mg/kg x 4 times). During the withdrawal phase, however, locomotor activity of drug-treated rats appears to return to that of the controls rather quickly. This recovery of the locomotor activity appears to reflect the normalization of DA level in the mesolimbic system; nevertheless, METH appeared to affect locomotor activity 3 days after the treatment, probably depleting DA in the NAc.

Effects of Methamphetamine and Phencyclidine on Social Interaction

METH and PCP differentially affected social interaction during the withdrawal periods: Days 3-7 (short-term) and Days 14-28 (long-term) of withdrawal. METH-treated rats showed a gradual decrease in social interaction on days 7 and 14 of withdrawal. On the other hand, PCP-treated rats showed no overall change in social interaction, except during the first 8 min observation period when they showed a decrease in social interaction across Days 7-28, with a greater decrease on Day 14. Further details of these drug effects are described below.

METH-treated rats showed comparable social interaction to that of saline-

treated rats during withdrawal Day 3. However, METH rats gradually decreased social interaction on Days 7 and 14, with a significant decrease on Day 14, and a return to the same level as control on Day 28. However, this is inconsistent with the previous finding that in adult rats, PCP decreased social interaction when tested 4 weeks after the last injection (Clemens et al., 2003). This difference may be due to the age and familiarity of the subjects. Developing rats with the same partners were used in the present study, whereas adult rats with different partners were used at each testing period in the previous study.

An interesting finding is that METH-treated rats gradually decreased social interaction on Day 7 and 14, with a significant decrease on Day 14, and returned to the same level as control on Day 28. Such recovery of social interaction during long-term withdrawal may reflect a transient change in neurotransmitter systems, possibly dopaminergic and serotonergic systems. In fact, change in the mesolimbic dopaminergic system, which consists of the nucleus accumbens (NAc), the prefrontal cortex (PFc), and the ventral tegmental area (VTA), has been implicated in regulation of social interaction (Tucci et al., 2000). In adult rats, dopamine was released from the VTA to the PFc and NAc while the animals were engaged in social interaction (Zhang et al., 1994), whereas loss of DA in the PFc decreased social interaction (Clemens et al., 2004; Espejo, 2003). These data indicate that enhanced dopamine in the mesolimbic system plays an important role in social interaction. In the present study, METH-treated animals showed a gradual decrease of social interaction on days 7 and 14 after the last injection. It is conceivable that such gradual decrease in social

interaction on Day 7 and 14 followed by recovery of social interaction on Day 28 may reflect a transient change in the mesolimbic system during short- and long-term withdrawal periods.

An alternative hypothesis emphasizes METH effects on the serotonergic system in the hippocampus (HIP), which may regulate anxiety. Social interaction has been frequently used to measure the effects of anxiolytic or anxiogenic drugs, with the assumption that a decrease in social interaction represents a state of high anxiety (File & Seth, 2002). Thus, it is reasonable to speculate about possible effects of METH on 5-HT and subsequent anxiety–related behaviors. For example, animals fed a tryptophan (a precursor of 5-HT)-depleted diet showed increased anxiety-related behaviors by spending more time in the corner of the openfield and by the wall, and such behavioral changes were correlated with decreased tryptophan in the HIP (Blokland et al., 2002). Similarly, in adult rats, pretreatment with 5-HT agonist into the HIP prevented anxiety-related behaviors (Kagamiishi et al., 2003). Although these findings provide strong support for the involvement of 5-HT in the anxiety-induced decrease in social interaction, there is a methodological complication. In these studies adult rats were paired with an unfamiliar partner at the time of testing, and their interaction was measured. In the present study, however, developing rats were raised in gang cages for 50 days (PD30-PD80, 4 rats/cage), during which time METH was administered and social interaction was measured. It is reasonable to assume that anxiety level due to encounter of new rats would be certainly higher than that due to interaction with familiar rats. Nevertheless, a possible role of reduced hippocampal 5-

HT in the gradual decrease in social interaction following METH treatment has not been ruled out.

Overall social interaction of PCP-treated rats was comparable to that of saline-treated rats throughout the withdrawal periods. However, PCP-treated rats showed a decrease in social interaction during the initial 8 min on Day 7, Day14, and Day 28 of withdrawal. Lack of PCP effects on overall social interaction is consistent with the findings of Sams-Dodd (2004) that in adult rats PCP failed to affect social interaction 10 days after the last treatment (Sams-Dodd, 2004). In an earlier study, Sams-Dodd (1996) reported that PCP decreased social interaction during the initial 10 min period in adult rats. The fact that locomotor activity was not affected on Day 7, Day 14, and Day 28 (see earlier discussion on Experiment 1) argues against the possibility of that the initial decrease in contact was caused by motor dysfunction. The present findings from juvenile rats and Sams-Dodd's report from adult rats indicate a similar change in social behavior during initial contacts. It is conceivable that a brief decrease in social contacts may be due to a social withdrawal effect of PCP.

PCP treatment in adulthood failed to affect social interaction in Sams-Dodd's study (2003), whereas PCP treatment during PD 50–51 produced a decreasing trend in social interaction in the present study. This discrepancy may be due to the age of the animals. According to Farber et al. (1995), sensitivity to the neurotoxic effects of NMDA antagonists in the limbic systems begins on approximately PD 45 and increases until PD 90-120. One explanation for the present findings is that NMDA blockade at PD 50–51 could have affected another neurotransmitter system, possibly

a dopaminergic system. In fact, exposure to NMDA blockade (1.25-5 mg/kg) in the early developmental period (PD1-PD21) altered the dopaminergic system in the PFc in adulthood (Wedzony et al., 2005). Given the evidence that the DA in the PFc plays an important role in social interaction (Clemens et al., 2004; Espejo, 2003), exposure to PCP during development may produce an enduring effect on social interaction by altering DA level in the PFc.

Decreased social interaction following PCP may be partly due to dysfunctional circuitry within the limbic system, particularly between the HIP and PFc. For example, PCP produces neural degeneration in the HIP (Ellison & Switzer 1993; Elllison et al., 1996) and neonatal lesions in the HIP decreased social interaction after maturation, while lesions in the PFC alone did not decrease social interaction (Flores, et al. 2005a), indicating that the pathway from the HIP to the PFc, but not from the PFC to HIP, mediates social interaction. This is consistent with the findings that the lesions in the HIP produced morphological change in the NAc and the PFc (Flores, et al. 2005b), and that simulation of the HIP increased DA in the NAc (Legault, et al. 2000; Floresco et al. 2001). Given the anatomical evidence that the NAc and PFc receive inputs from the HIP (Carr & Sesack, 1996; French & Totterdell, 2002), PCP may exert its effects by disrupting HIP function, which, in turn, affects the NAc and PFc, thereby decreasing social interaction.

Taken together, METH and PCP produced differential effects on social interaction during withdrawal periods. METH gradually decreased social interaction on days 7 and 14 after the last injection, while PCP decreased social interaction for an

initial 8 min during withdrawal periods. During withdrawal periods, METH appears to affect the mesolimbic system directly, while PCP appears to affect the mesolimbic system indirectly through the HIP. Differential effects of METH and PCP on social interaction during withdrawal periods may be due to the differential drug effects on different neurotransmitter systems and their interaction with the mesolimbic system.

Effect of Methamphetamine and PCP on Spatial Discrimination and Spatial Reversal

Effects of METH on Spatial Discrimination and Reversal. Rats treated with METH during PD 50-51 showed comparable performance on the spatial discrimination task, compared to that of the saline-treated rats when they were tested in adulthood (after PD 90). When these METH-treated rats were trained in a subsequent spatial reversal task, they showed a trend toward acquisition. Further details regarding the effects of METH on spatial discrimination are discussed below.

In the spatial discrimination task (SD), both METH- and saline-treated rats showed comparable performance, measured by the percent correct responses, response latencies, the number of barpresses during the inter-trial interval (ITI), and the number of days to reach behavioral criterion ($\geq$ 85 %, 3 sessions). During acquisition of SD, control and METH-treated animals took a similar number of sessions to reach a behavioral criterion and had a steady increase in the percent correct response, consistent correct response latency, a decrease in the incorrect

response latency, and a decrease in barpresses during the ITI. METH-treated rats showed no impairment that would reflect a long-term deficit in spatial learning, motor function, attention, or perseveration during acquisition of SD. Given that training on the SD began approximately 40 days after the last METH injection, the present findings indicate that there was no METH effect on SD. The present study is in agreement with a previous report that exposure to METH (5 mg/kg, 4 injections/day for 10 days) during PD 51-60 did not affect acquisition of a spatial water maze task 30 days after the last injection (Vorhees et al. 2005). Interestingly, however, METH (6.25 mg/kg/day, 4 injections/day for 10 days) given during PD 41-50 did impair acquisition of the spatial water maze task (Vorhees et al. 2005), suggesting that there is a time-window of sensitivity for METH effects. If this is the case, lack of METH effects on acquisition of the SD in the present study can be ascribed to METH treatment during a noncritical period during development. Nevertheless, the present findings suggest that exposure during development did not affect SD in adulthood.

In the subsequent reversal task (SDR), however, METH-treated rats tended to show a slower acquisition compared to saline-treated rats, especially toward the end of the training session. METH- and saline-treated rats showed similarities in response latencies, the number of barpresses during the ITI, and days to reach behavioral criterion. These results suggest that METH tended to decrease the accuracy in reversal learning, without producing motor deficits, attention deficits, or perseveration. The present study is inconsistent with the previous report that exposure to METH (5 mg/kg, 4 injections/day for 10 days) during PD 51-60 did not impair

reversal learning tested 30 days post injection (Vorhees et al. 2005). Given the comparability of the withdrawal periods used in the studies, the trend toward slowed reversal in the present study, but not in Vorhees et al. (2005) may be due to a difference in the tasks. In the reversal phase of the water maze task, animals were required to swim to the opposite side of the platform within 2 min per trial (Vorhees et al. 2005). During the reversal phase of the spatial discrimination task, animals were required to make the opposite barpress within 2 sec, demanding a greater ability to discriminate and to make correct responses within a short period of time. Thus, the reversal task employed in the present study may be more sensitive to the cognitive impairment.

A slow trend in acquisition of reversal task in METH-treated rats may be due to the dysfunctional state of the striatum and the medial prefrontal cortex. Recent study has suggested that in rats neurological changes in the striatum and the medial prefrontal cortex were closely associated with behavioral deficits during reversal learning (Daberkow et al. 2005; Kadota & Kadota, 2004). This is consistent with previous reports that the medial prefrontal cortex mediates reversal learning (Lacroix et al. 2002; Salazar et al. 2004). In particular, using the paradigm of the spatial discrimination tasks in the present study, Salazar et al. (2004) found that rats damaged in the medial prefrontal cortex showed a slower acquisition in the reversal task, without affecting initial acquisition of spatial discrimination. Taken together, in the present study, a slower learning seen in METH-treated rats during reversal may primarily reflect dysfunctional state of the medial prefrontal cortex.

Effects of PCP on Spatial Discrimination and Reversal. During the spatial discrimination task, PCP- and saline-treated rats showed a similar pattern of acquisition. During the reversal task, however, PCP-treated rats showed a retarded acquisition compared to the saline-treated rats. Further details are discussed below.

In the spatial discrimination task (SD), both saline- and PCP-treated rats had similar performance measures, including percent correct responses, response latencies, the number of barpresses during the inter-trial interval (ITI), and the number of days to reach a criterion (%CR $\geq$ 85%, 3 sessions). Across the training sessions, PCP- and saline-treated rats showed a steady increase in correct responses per session, and the groups showed no difference in the mean number of days required to reach a behavioral criterion. PCP- and saline-treated rats showed stable correct response latencies and a decrease in incorrect response latencies during acquisition of SD, indicating that there is no change in gross motor function. Moreover, both treatment groups showed a steady decrease in barpresses during the ITI, suggesting that PCP-treated rats did not exhibit 'impulsive' or 'disinhibitory' barpress responses during the course of SD acquisition. Thus, the present findings provide evidence that exposure to PCP (9 mg/kg, 12-hr interval, 4 injections) on PD 50-51 did not impair acquisition of spatial discrimination. This is in agreement with previous reports that in adult rats PCP and other similar non-competitive NMDA antagonists impaired spatial learning at acute and earlier withdrawal, but not over long-lasting, periods (Campbell et al., 2004; Kesner & Dakis, 1993; Whishaw & Auer, 1989). Such lack of effects on

learning during the withdrawal period is consistent with findings that in adult rats, PCP (5 mg/kg/day, 2 injections/day, 7 days) failed to affect acquisition of visual discrimination in the T-maze (Jentsch & Taylor, 2001) as well as odor and tactile discrimination after 10 days of withdrawal (Rodefer et al., 2005). However, it should be noted that PCP and MK-801, another non-competitive NMDA antagonist, impaired spatial learning during the acute drug phase and the early (Day 4) withdrawal period (Campbell et al., 2004; Kesner & Dakis, 1993; Whishaw & Auer, 1989).

Although the present findings from developing rats and previous findings from adult rats indicate that exposure (brief or long-term) to PCP does not affect acquisition of spatial discrimination, these findings are not in agreement with other reports, showing that PCP treatment during an earlier developmental period produces a long-lasting effect on spatial learning. For example, Sircar (2003) reported that exposure to PCP (5 mg/kg, once/day, 11 days) during development PD 5-15 impaired the acquisition of a spatial water maze task when the animals were tested in adulthood. Similarly, PCP (8.7 mg/kg, once/day, 3 days) treatment on PD 7, 9, and 11 produced a retarded acquisition of the spatial water maze task in adulthood (Wang et al. 2001). One explanation for such discrepancies between the present findings and Sircar's findings is that the age of the rats and the duration of the treatments differed: Sircar (2003) used PD 5-15 and PD 11 treatment days, whereas PD 50-51 and 2 treatment days were used in the present study. Thus, the present study gave evidence that PCP administered during the late developmental period does not affect spatial and

discriminative learning in adulthood.

In a subsequent reversal task (SDR), PCP-treated rats showed a characteristically different pattern of acquisition from that of saline-treated rats: They had a lower number of correct responses (i.e., a greater number of incorrect responses) and a greater number of barpresses during the ITI, particularly during the first half of acquisition, thereby yielding a retarded acquisition and requiring more days to reach a behavioral criterion (%CR >85%, 3 sessions). Both treatment groups showed similar patterns in other behavioral measures: stable correct response latencies and a decrease in the incorrect response latencies during acquisition of SDR, again indicating that there was no change in gross motor function. The difference did not seem to be due to a difference in motivational state because during the earlier stage of reversal PCP-treated rats tended to omit responding on a fewer number of trials (data not shown). Thus, the present findings provide evidence that exposure to PCP (9 mg/kg, 12-hr interval, 4 injections) on PD 50-51 selectively impaired reversal learning (SDR) without affecting the ability to learn a new task (SD).

Previous studies have indicated that PCP treatment affects the ability to inhibit the previously learned response. For example, Jentsch & Taylor (2001) reported that PCP (5 mg/kg, 2 injections/day, 7 days) impaired reversal learning in a visual discrimination task after 7 days of withdrawal. Interestingly, however, PCP did not affect the acquisition of a novel visual discrimination (Jentsch et al. 2001), suggesting that acquisition of a new task was not affected. These results are consistent with findings in the present study that exposure to PCP during PD 50-51 selectively

impaired reversal learning in adulthood, without affecting spatial discrimination.

Neurochemical and neuroanatomical changes produced by PCP may be responsible for enduring impairment in reversal learning in adulthood. According to Sircar (2003), rats receiving PCP on PD 5-15 showed upregulated NMDA receptors in the HIP and the frontal cortex in adulthood, suggesting that PCP treatment during early development produces long-lasting effects on spatial learning and spatial working memory in adulthood. Consistent with this notion, PCP on PD 7, 9 and 11 induced apoptosis in the frontal and olfactory cortices (Wang et al. 2001). Thus, exposure to PCP during early development would produce long-term impairment in spatial learning by producing neurochemical and neuroanatomical changes in the brain. Nevertheless, PCP treatment during later development and in adulthood fails to produce the same effect.

One line of evidence indicates that in adult rats blocking NMDA receptors in the HIP reliably impairs spatial learning (Kesner & Dakis, 1995; Kesner & Dakis, 1996). Microinjections of PCP or MK-801, NMDA antagonists, directly into the HIP disrupted spatial learning. Presumably, NMDA selectively impaired long-term memory by disrupting the consolidation process, while leaving short-term memory intact (Kesner & Dakis, 1995; Kesner & Dakis, 1996). These studies examined only the acute phase, and no long-term deficit in spatial learning was measured. Some studies have demonstrated that brief and chronic exposure to high doses of PCP produce neural degeneration in the limbic system, particularly the hippocampus (HIP), the retrosplenial cortex, and the posterior cingulate cortex (Ellison & Switzer 1993;

Elllison et al., 1996). Although PCP-induced behavioral deficits in the present study may reflect a dysfunctional state of the HIP in adulthood, the more pronounced deficit during the first half of reversal may reflect a transient dysfunction of the HIP. Perhaps previous reports of a lack of the long-lasting effect of PCP and other NMDA antagonists on spatial and discrimination learning (Jentsch & Taylor, 2001; Rodefer et al., 2005; Whishaw & Auer, 1989) are due not only to different behavioral measures and time of behavioral testing, but also due to differences in doses and frequency of administration, and the age of the rats. Also, enduring effects of PCP on learning in adulthood may depend on doses and frequency of administration during development. Thus, PCP-induced neurotoxic effects in the brain may be rather mild and transient, and thus insufficient to produce long-term effects on spatial learning in adulthood.

PCP-treated rats increased ITI barpresses during the first half of reversal (SDR). Interestingly, however, enhanced ITI barpressing was not observed during SD. In rats, the medial part of the prefrontal cortex (PFc) is thought to mediate reversal learning (Bussey et al. 1997). Excitotoxic lesions in the PFc produced retarded acquisition of a reversal task with impulsive responses occurring during the earlier phase of training session, yet the same animals showed normal acquisition of a spatial discrimination task using a visual stimulus (Salazar et al. 2004). Enhanced ITI barpresses may have reflected prefrontal dysfunction, particularly during reversal. This is consistent with the notion that repeated administration of PCP impairs rule-shift learning by damaging the dopaminergic system in the PFc (Jentsch & Taylor, 2001). Behavioral deficits in Salazar et al's study were similar to the present findings

that PCP-treated rats showed a selective impairment in reversal learning. However, PCP-induced deficits were due to dysfunction of PFc or HIP cannot be determined based on the present findings. Nevertheless, it is likely that PCP impairs the ability to shift rules, possibly by disrupting functions of the PFc.

Using brief exposure to PCP in development, the present study demonstrated a long-term deficit in reversal learning in adulthood. This may reflect the susceptibility of specific brain regions, such as PFc or/and HIP to PCP, as well as susceptibility of specific neurotransmitter systems, such as dopamine, during a critical period in development. Although it is inconclusive as to when such a critical period begins and ends, a few studies have demonstrated that NMDA antagonists begin to produce neurotoxicity in the limbic system approximately PD 45 and that toxicity increases until PD 90-120 (Farber et al. 1995; Farber, 2003). PCP administrations as well as learning tests were conducted in the present study during this time (PD90-120). Chronic NMDA antagonist (CGP 40116) administration over a 20 day period (PD 1-21) altered dopaminergic function in the PFc on PD 60 by reducing tyrosine hydroxylase by nearly 99% at the terminals in the PFc (Wedzony et al., 2005). Neuroanatomical studies have demonstrated that during development, dopaminergic fibers in the PFc increased in density through PD 20-60 and stops after PD 60 (Kalsbeek et al. 1988). Again, PCP treatment in the present study overlapped period between the onset of susceptibility to neurotoxicity and the last stage of the dopaminergic development (PD 45 and PD 60). Although, reversibility of the cognitive dysfunction induced by PCP is unknown (Jentsch et al., 2001), one would

predict that PCP treatment during this critical period may have produced irreversible effects on the dopaminergic neurons in the PFc, thereby producing long-term behavioral deficits in adulthood. One would also predict that the magnitude of the behavioral deficits would depend on the dose and frequency of PCP administration during this period.

General Discussion

The present study examined the effects of METH and PCP, given on PD 50-51 on locomotor activity, social interaction, and spatial and reversal learning. The findings in the present study indicate that the effects of METH and PCP on behavior differ depending on the complexity of the behavior as well as on the time of behavioral testing. METH and PCP affect behavior differently during the acute drug state as well as chronically. The present study focused on behavioral changes observed during the withdrawal period.

Clearly, METH and PCP affected locomotor activity during the acute drug state. An interesting finding was the way in which these drugs affected locomotion over time. The effects of acute METH on locomotor activity were characterized by a brief hyperlocomotion, followed by a sharp decrease in locomotion. After repeated administration, METH failed to affect locomotion during the acute drug state. Acute PCP effects on locomotor activity differed from acute METH effects. There was a steady increase in locomotion following acute PCP injection. After repeated

administration, a same dose of PCP further enhanced locomotion during acute drug state. Thus, with repeated administration, METH and PCP produced opposing effects on locomotion during the acute drug state. The differential behavioral changes immediately after METH and PCP are probably due to the different pharmacodynamics of each drug, particularly their action at target sites.

Drug effects on behavior were expected to become subtler during the withdrawal period (short- and long-term) compared to the acute drug state. Indeed, locomotor activity was not affected after 3 days of withdrawal. Although drug effects are rather subtle, difference in drug-induced behavioral changes appeared to linger after 14 days of withdrawal and last nearly 2 months after the last injection. During withdrawal Day 3, for example, social behavior of METH- or PCP-treated rats did not differ from that of control rats. On Day 7, however, drug-treated rats showed a decreasing trend in social interaction, compared to saline-treated rats that showed an increasing trend. By Day 14, overall social interaction differed between METH- and saline-treated rats: the METH group had a significant decline on withdrawal Day 14, while the control group had an increase. Such contrast in social interaction between METH-treated and control rats was not evident on Day 28. While the overall deficit in social interaction produced by METH was distinct, overall social interaction of PCP-treated rats was comparable to that of control rats. However, PCP-treated rats showed a decrease in their initial social interaction (8 min) on Days 7, 14 and 28. Such subtle deficits during an initial social encounter may reflect change in emotional state, such as anxiety.

The effects of METH and PCP on spatial learning differed, depending on the nature of the spatial task. Neither METH nor PCP affected spatial discrimination. During the spatial reversal task (SDR), however, METH-treated rats tended to show a slower acquisition of reversal, whereas PCP-treated rats showed a significantly impaired acquisition. Moreover, PCP-treated rats showed a high rate of barpress during the ITI, particularly during the early reversal phase. Thus, METH and PCP appear to produce characteristically different behavioral deficits during reversal.

It should be noted that testing of METH and PCP on spatial learning began after 4 weeks of withdrawal and lasted for 6 weeks, spanning a total of 10 weeks of withdrawal. , Comparing the present findings with other studies is rather difficult due to methodological differences, such as dose and frequency of drug injections, testing paradigm, and age of the rats. It is, however, reasonable to conclude that exposure to METH and PCP during later development produced differential effects on behaviors during the acute and withdrawal phases. Further studies on the distinctive changes produced by METH and PCP given at various developmental stages are warranted.

In summary, the present study provide strong evidence that brief exposure to METH and PCP during development acutely affects motor behavior and produces withdrawal effects on social behavior as well as enduring effects on complex learning. Exposure to METH and PCP affects higher order learning in adulthood. These changes were not detectable when a simpler behavioral measure, such as locomotor activity, was used. During development, the brain structures that mediate simple to higher functions undergo changes, and possibly have different sensitivities to METH

and PCP, yielding differential behavioral changes. During a critical period, the brain structures that mediate higher functions may be extremely vulnerable to neurochemical changes. Brief exposure to drugs, such as METH and PCP, during a critical period in development would produce profound change in these brain regions and produce enduring effects on higher cognitive function in adulthood.

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