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Brief Exposure to Methamphetamine (METH) and Phencyclidine (PCP) during Late Development Leads to Long-Term Learning Deficits in Rats

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Abstract

Exposure to methamphetamine (METH) and phencyclidine (PCP) during early development is thought to produce later behavioral deficits. We postulated that exposure to METH and PCP during later development would produce similar behavioral deficits, particularly learning deficits in adulthood. Wistar rats were treated with METH (9 mg/kg), PCP (9 mg/kg), or saline during later development, postnatal days (PD) 50–51, and subsequent behavioral changes were examined including: locomotor activity during the acute drug state (PD 50–51) and the post-drug phase (PD 50–80); social interaction on PD 54–80; and spatial discrimination and reversal in adulthood (after PD 90). METH and PCP differentially affected locomotion during the acute state, but not during the post-drug phase. METH decreased social interaction throughout tests two weeks after drug treatment, whereas PCP decreased social interaction only during the first 8 min of tests. Neither METH nor PCP impaired initial acquisition of spatial discrimination. However, reversal was significantly impaired by PCP, whereas METH produced a mild deficit, compared to controls. Our data provide evidence that exposure to PCP and METH during later development lead to enduring cognitive deficits in adulthood. Selective impairment of reversal may reflect neurological damage in the prefrontal cortex due to early exposure to drugs.

Keywords

developmental; dopamine; neurotoxicity; NMDA; prefrontal; reversal; social interaction

1. Introduction

Methamphetamine (METH) enhances dopamine and serotonin transmission in the brain (Friedman et al., 1998), whereas phencyclidine (PCP), an N-methyl-D-aspartate (NMDA) antagonist, disrupts glutamate transmission (Zukin and Javitt, 1993). Despite their action on different neurotransmitter systems, METH and PCP produce similar behavioral changes in adult rats. Acute injection of METH and PCP reliably enhanced locomotion and produced stereotypy at high doses (Clemens et al., 2004; Shoblock et al., 2003; Tanii et al., 1994; White et al., 1995). However, the effects of METH on locomotion lasted until 3 days, but not 7 days,

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after injection (Timar et al, 2003), whereas the effects of PCP lasted for 11 days (Sams-Dodd, 2004). Similar changes in behavior following METH and PCP are likely due to enhanced dopamine and glutamate in the mesolimbic system. Systemic injections of PCP increased glutamate and dopamine in the prefrontal cortex and the nucleus accumbens, and enhanced locomotion (Adams and Moghaddam, 1998; Javitt et al, 2004). Moreover, direct infusions of PCP into the nucleus accumbens or the prefrontal cortex enhanced locomotion and increased accumbal DA (Jentsch et al., 1998; McCullough and Salamone, 1992; Steinpreis and Salamone, 1993).

In developing rats, the effects of METH and PCP on behavior may differ, depending on the time of treatment and the type of behavioral testing. Studies have reported age-dependent effects of METH and PCP on locomotion during the acute and withdrawal phases. Acute effects of METH on locomotion have not been tested in adolescent rats, but, amphetamine, which has similar effects on the brain (Melega et al., 1995), enhanced locomotion on PD 18–22, but not on PD 34–38 (Lanier and Isaacson, 1977). Similarly, acute PCP on PD 5–40 increased locomotion, with a greater effect on PD 19 (Jacobs et al, 2000; Scalzo and Burge, 1994). During withdrawal, a decrease in locomotion was reported. METH treatment (30 mg/kg; PD 1–20) suppressed locomotion during withdrawal days 30–50 (Vorhees et al., 1994b). PCP treatment during PD 24–39 suppressed locomotion during days 10–40 of withdrawal, but such suppression was not seen if PCP was given during PD 4–19 (Scalzo, 1996), suggesting a greater susceptibility to the effects of PCP at a relatively older age. Thus, it appears that in developing rats, acute METH and PCP tend to increase locomotion, with a greater effect at younger age. Decreased locomotion seen during the withdrawal phase may depend on the time of drug exposure: a greater effect occurs when PCP, rather than METH, is given at older age. Such age-specific effects on locomotion may reflect a greater susceptibility to neurotoxicity induced by METH or PCP.

Social interaction in rats is often measured by the time spent in active interaction between two ‘unfamiliar’ rats and often includes behavioral categories such as sniffing, chasing, or grooming (Clemens et al., 2007, Tonissaa et al., 2004). The effects of METH or PCP on social interaction in developing rats are unknown. In adult rats, METH and PCP suppressed social interaction during acute and withdrawal phases (Clemens et al., 2004; McGregor et al., 2003; Sams-Dodd, 1998). According to Sams-Dodd (1996, 1998, 2004), acute injection of PCP decreases social interaction during the initial 10-min of encounter, but social interaction is restored after 10 days of withdrawal.

Learning appears to be differentially affected by METH and PCP, with a prolonged deficit in spatial learning following METH treatment, and a rather transient deficit following PCP. The effects of METH on learning were often tested during the withdrawal phase. In adult rats, METH treatment impaired performance in the object recognition task and a water maze task during 1–9 weeks of withdrawal (Bisagno et al., 2002; Friedman et al., 1998; Schroder et al., 2003). PCP acutely impaired spatial learning, but impairment abated during early withdrawal (Campbell et al., 2004; Kesner and Dakis, 1993; Whishaw and Auer, 1989). An interesting finding is that acute PCP selectively impaired set-shifting (Dalley et al., 2004; Rodefer et al., 2005) and reversal learning without affecting initial acquisition in adult rats (Jentsch and Taylor, 2001). Such selective deficits in reversal were interpreted as a lack of inhibition or flexibility in shifting rules (Dalley et al., 2004; Jentsch and Taylor 2001; Rodefer et al, 2005). These studies suggest that in adult rats the effects of PCP on learning may also depend on task specificity.

In developing rats, age-dependent learning deficits were reported following METH and PCP treatment. For example, repeated METH treatment during PD 11–20 impaired spatial learning assessed around PD 50 (after 4 weeks of withdrawal), but METH given on PD 1–10 failed to

do so (Vorhees et al., 1994a). Moreover, METH selectively impaired reversal learning without affecting working memory, only if METH was given during early (PD 11–15) or later development (PD 41–50), but not at other ages (Williams et al., 2003a, 2003b). Similarly, repeated PCP treatment on PD 5–15 impaired spatial learning after 3–6 weeks of withdrawal (Sircar, 2003), and it also impaired reversal learning in adulthood (Andersen and Pouzet, 2004). These studies suggest that in developing rats, repeated treatment with METH and PCP impairs learning during acute and extended withdrawal, and that such impairment depends on the specific time of exposure. Most developmental studies, however, have employed repeated treatment for 4–10 days during early development (before PD 20), and in some cases sub-chronic or chronic administrations (2–3 weeks) and relatively high doses were used. Thus, it is inconclusive as to when a critical period begins and ends, or if age-specific learning deficits demonstrated in these studies would attenuate. Nevertheless, an implication is that there exists a specific time-window when the nervous system may be vulnerable to drug-induced changes or neurotoxicity, leading to enduring behavioral deficits.

In the present study, we examined behavioral changes after treatment with METH and PCP during later development (PD 50–51), focusing on locomotor activity, social interaction, and visuospatial discrimination. To our knowledge, the behavioral consequences of brief drug exposure during later development have not been systematically examined. Given that NMDA antagonists begin to produce neurotoxicity in the limbic system around PD 45, possibly via a poly-synaptic chain of events (Farber, 2003; Farber et al., 1995; 2002), and that rapid changes occur in dopaminergic and glutamatergic systems during PD 40–60 (Anderson et al., 2000; Farber et al., 1995), our rationale was that even short exposure to METH or PCP during PD 50–51 would affect these neurotransmitter systems, leading to enduring deficits in a range of behaviors. We were particularly interested in the long-term behavioral consequences of brief, 2-day drug treatment. Adolescent rats (PD 50–51) were injected with METH (9 mg/kg), PCP (9 mg/kg), or saline, twice per day at a 12-hr interval for two consecutive days (a total of 4 injections). Drug effects were tested during the acute drug state (PD 50–51) and post-drug phase (PD 54–80) and in adulthood (after PD 80). We tested the hypotheses that: (1) METH and PCP would acutely increase locomotion but decrease it during the post-drug phase; (2) METH and PCP would decrease social interaction during the post-drug phase and would also impair learning in adulthood.

2. Results

2.1 Effects of METH and PCP on Locomotor Activity: Acute and Post-Drug Phase

Acute drug effects on locomotion were measured immediately after the first and the third injections. This testing schedule allowed us to test the rats twice at the same time of the day during the regimen of 4 injections at a 12-hr interval. Compared to saline, the first injection of METH and PCP significantly enhanced overall locomotion during a 60 min session (Fig 1A). Data in 5-min bins revealed a biphasic pattern after the first METH injection: locomotion peaked during the first 25 min, followed by a sharp decrease to the level of controls (Fig 1B). We did not measure stereotypy in this study. However, our informal observation was that during the second half of the session METH-treated rats showed stereotypy, characterized by repetitive head movement, sniffing, and rearing in adult rats (Castellani and Adams, 1981; Rebec et al., 1997; White et al., 1998). The first PCP injection produced an opposite pattern, with enhanced locomotion during the last 25 min of the session (Fig. 1B). A 3×12 ANOVA yielded a significant treatment effect [$F(2,28)=10.53$, $p<.01$], time effect [$F(11,308)=34.96$, $p<.01$], and interaction [$F(22,308)=12.88$, $p<.01$]. Pair-wise comparisons yielded a significant difference between METH and saline in each 5-min bin during the first 25 min [$t(21)=3.36, 7.17, 5.58, 3.49$, and 2.84 for five 5-min bins, $p<.01$], and between PCP and saline treatment during the last 25 min [$t(21)=3.12, 3.63, 5.11, 5.15$, and 5.16 for five consecutive 5-

min bins, $p < .01$]. The pattern was further differentiated after the third injection: locomotion was not affected by METH, but was further enhanced by PCP compared to the first injection (Fig 1C). A 3×12 ANOVA yielded a significant treatment effect [$F(2,28)=55.53$, $p < .01$], time effect [$F(11,308)=43.67$, $p < .01$], and interaction [$F(22,308)=4.81$, $p < .01$]. Pair-wise comparisons showed no differences between METH and saline groups ($t(21)=0.87, 1.01, 0.55, 0.12, 0.3, 0.46, 0.6, 0.74, 0.14, 0.69, 0.34$, and 0.45 for 12 consecutive 5-min bins, $p > .05$), suggesting that there was no change in locomotion throughout the session. Pair-wise comparisons showed significant differences between PCP and control in each 5-min bin throughout the session [$t(21)=4.71, 3.82, 5.58, 5.30, 6.98, 7.59, 9.48, 11.44$, and 9.98 for 12 consecutive 5-min bins, $p < .01$].

Post-Drug Phase—Locomotor activity of METH and PCP groups was comparable to that of the control during the post-drug phase: Day 3, 7, 14, and 28 (Fig. 2A–D). A 3×12 ANOVA yielded no significant treatment effects [$F(2,28)=0.32$, $p > .05$]. There was a significant effect of time on Day 3 [$F(11,308)=67.09$, $p < .01$], Day 7 [$F(11,308)=56.57$, $p < .01$], Day 14 [$F(11,308)=51.42$, $p < .01$], and Day 28 [$F(11,308)=83.46$, $p < .001$], with no significant interaction on Day 7, 14, and 28 ($p > .05$). A significant interaction [$F(22,308)=1.62$, $p < .05$] on Day 3 was due to a difference between METH and controls during the first [$t(21)=2.68$, $p < .05$] and the fifth 5-min bins [$t(21)=3.31$, $p < .01$].

We monitored the body weight of each rat closely from habituation to the early post-drug phase, particularly during the first week of the experiment. Prior to habituation, the mean body weights of the METH (188.6 g), PCP (189.9 g), and control groups (189.4 g) were comparable. However, during the post-drug phase, each group gained weight at a different rate: on average the METH group gained 7.1 g per day, the PCP group 8.6 g per day, and control rats 10.2 g per day. Differences in body weight were not observed four weeks after drug injection. Given that these rats were housed together in group cages, the slower weight-gain of drug-treated rats during the early post-drug phase may be ascribed to side-effects associated with METH and PCP.

2.2 Effects of METH and PCP on Social Interaction during Post-Drug Phase

Social interaction was tested during the post-drug phase: 3, 7, 14, and 28 days after the last treatment. Social interaction of saline- and drug-treated rats was scored separately, by alternating 4 min intervals within each 8-min observation period. Scores were calculated by multiplying frequency and duration in each 8-min interval. Overall social interaction was averaged across the session. This was similar to behavior scoring methods used in previous studies (Fone et al., 2002; White et al., 1995). The METH group showed a gradual decrease in social interaction from Day 7 to a low on Day 14, compared to control rats that showed a gradual increase in social interaction from Days 3 to 14 (Fig. 3A and 3C). A 2×4 ANOVA yielded a significant interaction between treatment and days [$F(3,36)=4.53$, $p < .01$], but no significant treatment effect [$F(1,12)=0.59$, $p > .05$] or day effect [$F(3,36)=0.50$, $p > .05$]. Pairwise comparison showed a significant difference between METH and saline treatment 14 days after the last injection [$t(12)=2.32$, $p < .05$].

Overall social interaction of the PCP group was similar to that of controls on 3, 7, 14, and 28 days after the last injection (Fig 3B, top). A 2×4 ANOVA showed no significant treatment effect [$F(1,10)=0.03$, $p > .05$] or interaction [$F(3,30)=0.39$, $p > .05$], but showed a significant effect of days [$F(3,30)=8.44$, $p < .01$]. Guided by Sams-Dodd's report (1996) that in adult rats PCP decreased social interaction during the initial 10-min interaction between two unfamiliar rats, we further analyzed the first 8-min of social interaction. We found that compared to controls, the PCP group showed a decreased social interaction during the first 8-min on Days 7, 14, and 28 (Fig. 3B, bottom). A 2×4 ANOVA showed a significant treatment effect [F

(1,10)=5.65, $p<.05$], but no significant effect of days [$F(3,30)=0.17$, $p>.05$] or interaction [$F(1,10)=0.77$, $p>.05$]. Pair-wise comparisons showed no significant group difference between testing sessions ($p>.05$). To rule out the possibility that locomotor side-effects of PCP impaired social interaction, we further analyzed the first 8 min of locomotor activity during days 3, 7, 14, and 28 of the post-drug phase. A 2×4 ANOVA on 8 min locomotor activity showed a significant effect of days [$F(3,63)=2.93$, $p<.05$]; but no significant treatment effect [$F(1,21)=0.22$, $p>.05$] or interaction [$F(3,63)=1.42$, $p>.05$]. Thus, decreased social interaction in the PCP group during the initial encounter was not due to a change in locomotor activity.

2.3. Effects of METH and PCP on Spatial Discrimination and Reversal in Adulthood Spatial discrimination (SD)

The long-term consequences of METH and PCP treatment during PD 50–51 were tested with spatial discrimination in adulthood, after PD 90. During acquisition, both METH and control groups showed comparable performance on all measures, with a steady increase in the %CR across training sessions (Fig. 4A). There was a significant effect of training [$F(21,315)=79.68$, $p<.01$], but no treatment effect [$F(1,15)=0.31$, $p>.05$] or interaction [$F(21,315)=0.60$, $p>.05$]. Days to reach a criterion was similar in both groups [$F(1,15)=1.79$, $p>.05$] (Fig. 4A, Inset). Correct response latencies were stable across sessions in both groups (Fig. 4B, Top), with no treatment effect [$F(1,15)=1.13$, $p>.05$] or training effect [$F(21,315)=1.44$, $p>.05$], but a significant interaction [$F(21,315)=1.87$, $p<.05$] due to shorter latencies in the METH group in sessions 15 and 19 ($t(15)=1.88$ and 2.16 , respectively; $p<.05$). Incorrect response latencies steadily decreased in both groups (Fig. 4B, Bottom), as indicated by a main effect of training [$F(21,315)=12.94$, $p<.01$], but no treatment effect [$F(1,15)=0.34$, $p>.05$] or interaction [$F(21,315)=0.46$, $p>.05$]. Barpresses during the ITI decreased with training in both groups (Fig. 4C) [$F(21,315)=48.06$, $p<.01$], with no treatment effect [$F(1,15)=0.36$, $p>.05$] or interaction [$F(21,315)=0.92$, $p>.05$].

The PCP group's performance was similar to controls on most measures: Both groups showed a gradual increase in %CR with training (Fig. 5A) and required a similar number of days to reach a criterion (Fig. 5A, Inset) [$F(1,15)=1.31$, $p>.05$]. A 2×22 ANOVA yielded a significant training effect [$F(21,315)=113.74$, $p<.01$], but no treatment effect [$F(1,15)=1.44$, $p>.05$] or interaction [$F(21,315)=1.08$, $p>.05$]. Although both PCP and control groups showed stable correct response latencies across sessions (Fig 5B, Top), with no treatment effect [$F(1,15)=0.08$, $p>.05$] or interaction [$F(21,315)=0.48$, $p>.05$] and a significant effect of training was obtained [$F(21,315)=2.35$, $p<.01$]. Incorrect response latencies steadily decreased with training in both groups (Fig. 5B, Bottom) [$F(21,315)=21.31$, $p<.01$], with no treatment effect [$F(1,15)=1.51$, $p>.05$] or interaction [$F(21,315)=0.48$, $p>.05$]. In both groups, the ITI barpresses also decreased gradually with training [$F(21,315)=69.01$, $p<.01$], with no treatment effect [$F(1,15)=0.05$, $p>.05$] or interaction [$F(21,315)=1.33$, $p>.05$] (Fig 5C).

Reversal of Spatial Discrimination (SDR)—Reversal training began after the animals' performance on SD reached the behavioral criterion. During reversal the METH group tended to show a slightly slower increase in %CR compared to saline-treated rats (Fig. 6A). A 2×22 ANOVA showed a trend toward a treatment effect [$F(1,15)=3.30$, $p=0.06$] and a main effect of training [$F(21,315)=54.17$, $p<.001$], but no significant interaction between treatment and training [$F(21,315)=0.61$, $p>.05$]. Days to criterion did not differ (Fig. 6A, Inset) [$F(1,15)=2.11$, $p>.05$]. Although both group took a comparable number of days to acquire SDR, the METH group tended to show a lower %CR during the later training sessions. Pairwise comparisons showed significant differences in %CR between METH and control groups on training sessions 6, 12, 18, 20, 21, and 22 [$t(15)=2.37$, 2.17 , 2.15 , 2.14 , 2.31 , and 2.38 , respectively; $p<.05$]. The correct response latency was similar in both groups (Fig. 6B, Top), with no treatment effect [$F(1,15)=0.01$, $p>.05$] or interaction [$F(21,315)=0.56$, $p>.05$]. A

training effect was obtained [$F(21,315)=2.03, p<.01$]. Incorrect response latency was also similar in both groups, with a steady decrease across sessions (Fig. 6B, Bottom). A 2×22 ANOVA yielded a training effect [$F(21,315)=8.17, p<.001$], but no significant treatment effect [$F(1,15)=0.05, p>.05$] or interaction [$F(21,315)=0.80, p>.05$]. The ITI barpresses decreased with training in both groups [$F(21,315)=9.60, p<.001$], without treatment effect [$F(1,15)=0.49, p>.05$] or interaction [$F(21,315)=0.32, p>.05$] (Fig. 6C).

PCP treatment on PD 51–52 significantly affected reversal learning in adulthood, with a slower increase in %CR compared to controls (Fig. 7A) and a greater number of days to reach a behavioral criterion compared to controls (Fig. 7A, Inset) [$F(1,15)=6.53, p<.05$]. A 2×22 ANOVA yielded a significant treatment effect [$F(1,15)=4.54, p<.05$], and performance differed on training sessions 6, 10, 12, 15, 18, and 20 [$t(15)=2.44, 2.16, 2.58, 3.43, 2.26$, and 2.72 , respectively; $p<.05$]. There was a significant effect of training session [$F(21,315)=117.94, p<.001$], but no significant interaction [$F(21,315)=0.69, p>.05$]. Correct response latencies of the PCP and control groups did not differ (Fig. 7B, Top), with no effects of treatment [$F(1,15)=1.67, p>.05$], session [$F(21,315)=1.22, p>.05$] and no interaction [$F(21,315)=1.38, p>.05$]. Both groups showed a steady decrease in incorrect response latencies with training (Fig. 7B, Bottom) [$F(21,315)=7.82, p<.001$], with no treatment effect [$F(1,15)=0.57, p>.05$] or interaction [$F(21,315)=0.66, p>.05$]. An interesting finding was that the PCP group made more barpresses during the ITI than control rats, particularly during the earlier phase of reversal (Fig. 7C). A 2×22 ANOVA yielded significant effects of treatment [$F(1,15)=5.93, p<.05$], session [$F(21,315)=58.54, p<.001$], and an interaction [$F(21,315)=3.89, p<.001$]. Pair-wise comparison yielded differences between PCP and control groups for sessions 1, 2, 3, 5, 6, and 19 [$t(15)=2.64, 3.24, 2.46, 2.15, 2.26$, and 2.23 , respectively; $p<.05$].

3. Discussion

Effects of METH and PCP on Locomotor Activity

During the acute state, METH and PCP produced distinctive effects on locomotion, with marked changes with METH and progressive changes following PCP. After the first METH injection, hyperlocomotion peaked during the first 30 min, followed by a sharp decrease, whereas the third METH injection failed to increase locomotion. PCP's effects on locomotion were opposite: the first PCP injection gradually increased locomotion towards the second half of the session, whereas the third PCP injection produced a continuous increase throughout the session.

A biphasic pattern of locomotion in the METH group (9mg/kg) was similar to a previous report that METH (3–20 mg/kg) produced a brief increase in locomotion followed by a decrease (Mori et al., 2004). According to Shoblock et al (2003), METH (2 mg/kg) produced continuous hyperlocomotion and increased DA in the nucleus accumbens 20–40 min after injection. A shorter time course of hyperlocomotion in our study was likely due to a dose difference: a high dose (9 mg/kg) of METH led to a rapid decrease in locomotion due to the emergence of stereotypy. Our informal observation suggested that acute METH elicited 'stereotypies' characteristics of adult rats, including repetitive head movement, sniffing, and rearing reported in adult rats (Rebec et al, 1997; White et al., 1995, 1998). Consistent with a previous report that PCP (2.5–10 mg/kg) enhanced locomotion in a dose-dependent manner (Tanii, et al., 1994), PCP-treated rats in our study showed a steady increase in locomotion towards the second half of the session (approx. 25 min after treatment), which corresponded with the reported time course of increased DA in the nucleus accumbens and the striatum, 20–40 min after PCP (2.5–10 mg/kg) (Hanson et al., 1995; Nishijima et al., 1996). Clearly, our data indicate that there was a carryover effect across the first three injections in both METH and PCP groups. Nevertheless, the third injection further dissociated the effects of the drugs on locomotion. In the METH group, carryover reduced locomotion due to competing stereotypic behaviors,

whereas in the PCP group it resulted in a sustained hyperlocomotion. Given the evidence that behavioral excitation reflects increased DA transmission in the nucleus accumbens (Tran et al., 2004), and that METH (4.8 mg/kg) increased peak accumbal DA nearly fivefold compared to PCP (10 mg/kg) (Shimada, et al., 1996), a lack of locomotion in the METH group in our study may have been due to a sustained level of excessive accumbal DA, whereas sustained hyperlocomotion in the PCP group may have been due to a continuous increase in accumbal DA.

During the post-drug state (Days 3–28), neither METH nor PCP affected locomotion 7–28 Days from the last injection. On Day 3, the METH group briefly decreased locomotion during the first 5 min, but normalized quickly. Timar et al (2003) reported that locomotion decreased 3 days after METH (10 mg/kg, 4 treatments, 2-hr interval), but normalized during testing periods similar to ours. In their study, locomotion was measured in a novel environment, whereas our rats were well habituated, so the time course of normalization was similar even though the frequency and interval of injection as well as the testing environment differed. Locomotion of the PCP group was comparable to that of controls 3–38 days after injection. The dose and frequency of PCP injection in our study were less than half of that used in Hanania et al's study (1999) which reported PCP (20 mg/kg/day, 5 days) produced sensitization 3 and 8 days after injection when animals were challenged with 3.2 mg/kg. We did not measure accumbal dopamine and do not know how quickly the normal level of DA would be restored during the post-drug phase. However, in light of evidence that sensitivity of accumbens neurons returned to normal within 10 days after METH (5 mg/kg, 5 injections/day) (Amano et al., 1996), a lack of drug effect on locomotion 3–28 days after METH and PCP in our study may reflect normalization of accumbal DA and possibly a slightly earlier restoration of accumbal DA following PCP.

Effects of METH and PCP on Social Interaction during the Post-Drug Phase

Social interaction measured during the post-drug phase, Days 3, 7, 14, and 28, was differentially affected by METH and PCP. On Day 3, social interaction of the METH group was similar to that of control rats, but it gradually decreased on Days 7 and 14, with a significant decrease on Day 14, and it returned to the control level on Day 28. An interesting trend was that social interaction of control rats continued to increase until Day 14, whereas that of METH rats continued to decrease. Given that locomotor activity was restored by Day 7 (see above), the lack of group difference in social interaction on days 7 and 28 suggests that it is unlikely that a gradual decrease in social interaction was due to possible side-effects of METH, such as slow weight-gain or ataxia. In adult rats, social interaction is often measured by the time spent in active interaction between two 'unfamiliar' partners (Clemens et al., 2004; 2007, Tonissaaar et al., 2004). In adult rats, chronic or intermittent METH treatment decreased social interaction and prefrontal DA 4–7 weeks after the last injection (Clemens et al., 2004; 2007). Our rats were housed in group cages on PD 30–80, during which time METH was administered and social interaction was measured. Thus, differences between the reports of Clemens' group and ours could be due to the age of the rats and their familiarity with each other. Alternatively, social interaction in the METH group may have been due to increased anxiety. A state of high anxiety decreases social interaction (File and Seth, 2002) and increases time spent in the corner of the openfield (Blokland et al., 2002). Although it is unlikely that an encounter with familiar rats, as in our study, would produce a high level of anxiety, the possibility that drug-induced anxiety decreased social interaction cannot be ruled out.

The PCP group showed a decrease in social interaction only during the first 8-min encounter in a 60-min session, but no overall change was found on Day 7, Day 14, and Day 28 after drug treatment. Our rats were well habituated in the openfield, and the decreased interaction during the first few min was probably not due to novelty, a non-specific decrease in activity, or a gross

motor deficit. Similarly in a previous study, overall social interaction between unfamiliar adult rats was not affected by PCP, but their initial 10-min interaction was decreased 10 days post-injection (Sams-Dodd, 1996; 2004). One remaining issue was that the pattern of interaction among rats in the PCP group differed from that of the METH group. According to Tonissaar et al (2004), the social interaction time of rats was closely correlated with their partner's social activity during testing. Perhaps, PCP promoted differential interaction of the four 'familiar' rats in their home cage and this affected social activity in the openfield. Given that social interaction and prefrontal DA are closely linked (Espejo, 2003; Tucci et al., 2000), and that prefrontal DA increases during social interaction (Zhang et al., 1994), future study should examine whether the gradual decrease in social interaction in the METH group on days 7 and 14, followed by recovery on Day 28, reflected a transient change in prefrontal DA.

Effect of METH and PCP on Spatial Discrimination and Reversal in Adulthood Spatial Discrimination (SD)

After PD 90, approximately 40 days after the last drug injection, rats were trained in SD and a subsequent reversal. During acquisition, METH- and saline-treated rats showed comparable performance: a steady increase in the percent correct response (%CR), a similar number of sessions to reach a behavioral criterion, stable correct response latencies, and a gradual decrease in the incorrect response latency and the ITI barpresses. These results suggest that there was no obvious long-term METH effects on SD learning, motor function, or perseveration. Our results are similar to a previous report that METH (5 mg/kg, 4 times/day) on PD 51–60 did not impair acquisition of a water maze task when testing occurred 30 days after the last injection (Vorhees et al. 2005). Interestingly, METH (6.25 mg/kg, 4 times/day) on PD 41–50 impaired acquisition of the same water maze task (Vorhees et al. 2005), suggesting that there is a critical time-window for METH to have adverse effects. If this is the case, lack of METH effects on SD acquisition in our study can be ascribed to METH exposure during a noncritical period of development.

PCP treatment did not impair acquisition of SD in adulthood. Performance of the PCP and control groups was similar: a steady increase in correct responses, a comparable number of days to reach a behavioral criterion, stable correct response latencies, and a gradual decrease in incorrect response latencies and ITI barpresses. A lack of PCP effects on SD is consistent with a previous report that in adult rats, PCP (5 mg/kg/day, twice/day, 7 days) failed to affect acquisition of visual discrimination in the T-maze (Jentsch and Taylor, 2001) and odor or tactile discrimination 10 days after injection (Rodefer et al., 2005). Previous reports indicate that PCP and other similar NMDA antagonists impaired spatial learning during early withdrawal (day 1–4), but not after a longer term (Campbell et al., 2004; Kesner and Dakis, 1993). Moreover, either PCP (5 mg/kg, once/day) on PD 5–15, or PCP (8.7 mg/kg, once/day) on PD 7–11 impaired the acquisition of a water maze task in adulthood (Sircar, 2003; Wang et al., 2001). The discrepancy between these studies and our findings is likely due to the age difference of subjects (early vs. late development) and the treatment frequency and duration (11 days vs. 2 days).

Reversal Task (SDR)—During reversal, the METH group tended to show a slower acquisition toward the end of training, compared to controls. Response latencies, ITI barpresses, and days to criterion were comparable in both groups. Our findings suggest that METH impairs accuracy during SDR, without affecting other behavioral measures. According to Vorhees' group, METH given during PD 51–60 did not impair reversal learning tested 30 days post injection (Vorhees et al., 2005). Given that the age of the subjects and the duration of withdrawal in their study and our study were comparable, this discrepancy is likely due to a dose difference: 5 mg/kg (4 times/day, 10 days, used in Vorhees' study) vs. 9 mg/kg (twice/day, 2 days, used in our study). Another possibility is the task difference: reversal of the water

maze task required that the animal to swim to the opposite side of the platform within 2 min per trial (Vorhees et al., 2005), whereas our reversal task required the animal to make a response within 2 sec and involved 100 rewards per 60-min session.

PCP impaired performance during SDR. The PCP group showed a greater number of errors and barpresses during the ITI, particularly during the first half of acquisition, but no change in response latencies, yielding retarded acquisition and resulting in more days to reach a criterion (%CR >85%, 3 sessions). Our results provide evidence that exposure to PCP during later development selectively impaired reversal learning in adulthood, without affecting the ability to learn a new task. Similarly, in adult rats, PCP did not affect acquisition of a novel visual discrimination 7 days after the last PCP injection, but impaired reversal of the same task (Jentsch and Taylor, 2001). These results suggest that regardless of age, PCP impairs the ability to inhibit the previously learned response, which is specific to reversal learning.

An interesting observation was that during the early half of SDR acquisition, the PCP group made a greater number of barpresses during the ITI. This appears to be specific to reversal because during the SD the number of ITI barpresses of the PCP group did not differ from that of controls. This also suggests that impaired performance is not due to a change in motivational state. Previously, we reported that excitotoxic lesions in the prefrontal cortex retarded reversal, with animals making impulsive responses during the early phase of reversal, without affecting acquisition of initial spatial discrimination (Salazar et al., 2004). In the present study, we reversed the sequence of behavioral tasks: the SD used in the previous study (Salazar et al., 2004) was used as the reversal task and vice versa. The SD in the present study was more difficult than the SDR. Nevertheless, we found a similar behavioral impairment, a selective deficit in reversal in the PCP group. Although it is inconclusive as to when a critical period begins and ends, NMDA antagonists may begin to produce neurotoxicity around PD 45 (Farber et al., 1995). Our adolescent rats were exposed to PCP on PD 50–51, a period of susceptibility to toxicity, and they showed a selective impairment on SDR in adulthood (PD90–120): This impairment may reflect irreversible damage in the prefrontal cortex. Our data support the notion that targeting glutamatergic function may prevent against damage by PCP (Harich et al, 2007, 2008).

In summary, we provide evidence that the way in which brief exposure to METH and PCP during development affected behavior differed over time, and that drug effects depended on the nature and complexity of behavior. METH and PCP markedly affected locomotion during the acute state, but not during the post-drug phase. Towards the second week of the post-drug phase, the METH group decreased social interaction, whereas the PCP group showed a minimal decrease during initial encounter. When learning ability was tested in adulthood, the PCP group's performance in reversal was markedly impaired, particularly during the early phase, whereas the METH group showed a minimal impairment in reversal. Neither group showed impairment in SD, the initial learning task. Clearly, brief exposure to METH and PCP on PD 50–51 leads to enduring deficits on higher cognitive function in adulthood. It is not clear whether exposure to these drugs during later development is more detrimental than exposure during early development. Although there has been alarming report of intermittent or frequent use of drugs among adolescents, little is known about the long-term consequences of intermittent use of drugs, such as METH. Following a 2-day exposure to high doses of METH or PCP, we systematically examined different behaviors from adolescence to late adulthood. Our results provide evidence that even brief exposure to drugs during adolescence or young adulthood is likely to produce enduring learning deficits. Further studies on drug-induced changes in specific brain regions that mediate higher functions and on regional differences in sensitivity to METH and PCP across different stages of development are warranted.

4. Experimental Procedures

4.1. Animal Preparation and Drug Administration

Male Wistar rats (PD 30–150; n=120) were housed in the animal colony at the Psychology Department, Morehead State University, under a 12/12 light-dark cycle. Rats (PD 30; Harlan, Indianapolis, IN) were received and adapted to the colony environment three week prior to the start of the experiment. Prior to the experiment all rats were handled for 5 min per day for at least 3 days. Rats were kept in group cages (4 rats/cage) until PD 80, with free access to food and water. In the *Locomotor Activity* experiment, two of four rats in each group cage were color coded using permanent markers: either red (drug-treated) or black (saline-treated). In the *Social Interaction* experiment, four rats in each group cage were color coded: red throughout the length of the dorsal surface (drug), black at the posterior part of the dorsal surface (saline), green at the center part of the dorsal surface (no injection), and yellow at the anterior part of the dorsal surface (no injection). In the *Learning* experiment, rats were separated into single cages after PD 80 and were food-deprived (85% of free feeding weight) for training. Water was available *ad libitum*. All experimental procedures and animal care were in accordance with the NIH guidelines and were approved by the Institutional Animal Care and Use Committee.

d-Methamphetamine HCl (METH; Sigma, St. Louis, MO) and phencyclidine (PCP; Sigma) were dissolved in saline and administered on PD 50–51. METH (9 mg/kg) and PCP (9 mg/kg) were given twice/day (9 mg/kg/injection, a total of 18 mg/kg/day at a 12-hr interval) for two consecutive days (a total of 4 injections). Saline was used as a control. All injections were given subcutaneously (sc). The animals used in all experiments were exposed to only one treatment condition. Treatment regimen (dose, frequency, and interval) was identical in all experiments. A dose of 9 mg/kg of METH and PCP was used because this dose was at the lower end of those that produced long-term changes. Sub-chronic injections of 15 mg/kg of METH (each given twice per day; PD 11–20) led to impairment in learning (Vorhees et al., 1994a). Similarly, 10mg/kg of METH (4 injections/day, PD 41–50) is thought to produce toxicity and impaired spatial learning in adulthood (Vorhees et al., 2005; Williams et al., 2003a). Similarly, in adult rats METH (4 injections of 12.5 mg/kg at a 2 hr interval) impaired learning 65 days later (Friedman et al., 1998). One-day treatment with METH, either 10–15 mg/kg (4 injections, each given 2 hrs apart) or 30 mg/kg (single injection) produced neurotoxicity and apoptosis within 24–48 hrs in mice (Fumagalli et al., 1998; Zhu et al., 2005a; 2005b). Moreover, NMDA antagonists begin to produce neurotoxicity in the limbic system around PD 45, possibly via a poly-synaptic chain of events (Farber et al. 2002; Farber, 2003). PCP (5–20 mg/kg/day, via minipump, for 2 weeks) affected prefrontal DA receptors, but changes induced by PCP (15 mg/kg/day) were restored after 4 days of withdrawal (Balla et al, 2003), the time at which locomotor activity was tested in the present study. Although PCP (5–30 mg/kg/day for 6 consecutive days) administered at 3 months of age failed to affect social interaction, PCP (50 mg/kg, 1–4 injections) produced neurotoxic effects (Sams-Dodd, 2004).

In this study, we used the term ‘late development’ to refer to the age PD 45–60, and rats PD 50–51 were tested as ‘adolescent’ and PD 80 or older as ‘adult’. Researchers have disagreed about the age at which rats should be considered ‘adolescent or juvenile’ is inconsistent, ranging from PD 28- around PD 44 (Martinez et al., 2000; Sircar and Rudy, 1998). Likewise, rats ranging from PD 60–90 have been considered ‘young adult’ or ‘adult’ (Kokoshka et al, 2000; Martinez et al., 2000; Sircar, 2003). The ages at which we gave drugs and the duration of administration were based on developmental changes. For example, NMDA antagonists begin to produce neurotoxicity in the limbic system at approximately PD 45 and continue until PD 90–120 (Farber et al. 1995; Farber, 2003). The density of dopamine fibers in the prefrontal cortex and the striatum increases during PD 40–60 and stops increasing after PD 60 (Kalsbeek et al. 1988). Similarly, density of DA receptors in the prefrontal cortex peaks around PD 40–

60 (Anderson et al., 2000) and peaks around PD 40 in the striatum peaked and then declines until PD 120 (Gelbard et al., 1989). Although it is inconclusive as to when such a critical period begins and ends, at a minimum, drug treatment given on PD 50–51 in the present study overlaps a period of susceptibility to drug-induced changes. From the standpoint of the development of the dopaminergic and the glutamatergic systems, PD 50–51 is a reasonable time-window within which to produce an impact of brief exposure and to systemically assess the later consequences of exposure on behavior.

4.2. Behavioral Procedures

Locomotor Activity—Rats (n=32) were randomly assigned to one of four open-fields (76 × 76 × 40 cm). Locomotion was measured by distance traveled using a video tracking system (San Diego Inst, USA). Output from a video camera mounted on the ceiling centered above the open-fields was routed to a computer, which captured the contrast between the light subject (rat) and the gray background at a speed of 30 images/sec. Locomotion was measured every 5 min in a 60-min session during three phases: habituation, acute drug state, and post-drug phase. A 60-min habituation was done 24 hrs prior to drug phase. Habituation minimized handling-related dopamine release (Feenstra, 2000; Feenstra et al., 2000), rearing (isolation) conditions, or novelty-related behavioral changes, which may lead to increased or decreased exploratory locomotor behavior (Burns et al., 1996; Legault and Wise, 2001; Levine et al., 2008). Our rats were handled, group-reared, and habituated in the openfield prior to experiment. In our study, locomotion was measured by distance traveled, and ataxia, such as uncoordinated, awkward, or jerky movements, associated with acute PCP (Sircar and Soliman, 2003; White et al., 1995) was not excluded from 'locomotion'. Rats received a total of 4 injections with each injection being 12-hrs apart across two consecutive days. Testing during the acute drug state was done immediately after the first and the third injections. Behavioral testing during the post-drug phase was done 3, 7, 14, and 28 days after the last injection, which corresponded to PD 54, 58, 65 and 79. Behavioral testing was done during the light phase (1 session/day). After each session, rats were returned to their group cages.

Social Interaction (SI)—Rats (n=56; 4 rats/squad; 14 squads) were habituated to the open-field for 60 min prior to testing. Rats were divided into two treatment conditions: PCP (n=28; 4 rats/squad; 7 squads) and METH (n=28, 4 rats/squads; 7 squads). Social interaction (SI) was tested during the post-drug phase, 3, 7, 14 and 28 days after the last injection. Four groups (one group/open-field) were taped simultaneously for 60 min for off-line analysis. The 60-min session was divided into 7 segments (8 min/segment), excluding the first 2 min. During each segment, social interaction of saline- and drug-treated rats was scored separately, by alternating 4 min intervals within each 8-min observation period in a counter-balanced manner. SI was defined as occurrence of any of three behaviors: (1) locomotion toward another rat to make contact (active approach); (2) turning the head toward another rat (> 45° and making contact); or (3) maintaining physical contact with any rat. Frequency and duration of each behavior in a given observation period were divided into five intervals. For frequency these were 0–4 where 0 = 0; 1 = 1; 2–5 = 2; 6–13 = 3; > 13 = 4. For duration these were 0–4 where 0 sec = 0; 1–86 sec = 1; 87–159 sec = 2; 160–238 sec = 3; >238 sec = 4 for duration. SI scores were calculated by multiplying frequency and duration in each observation period, and they were averaged across the entire session. This is similar to behavior scoring methods used in previous studies (Fone et al., 2002; White et al., 1995). The distribution of multiplied scores (frequency × duration) yielded a standard normal curve. Pearson correlation showed 96% intra-observer reliability in scoring of frequency and 99% in the scoring of duration. It should be noted that our measure of 'social' interaction focused on interaction of a focal rat (drug or vehicle-treated) with 'familiar' rats housed together until PD 80, and differed from that between two 'unfamiliar' adult rats reported in previous studies (File and Seth, 2002; Tonissaar et al.,

2004). Our particular interest was the effects of drugs on social interaction in a familiar environment.

Spatial Discrimination and Reversal—Each operant chamber (Coulbourn Inst.) was placed in a sound-attenuating box and was equipped with a house light, two levers, cues lights above each lever, a speaker, and a pellet dispenser located between the levers. Initially, rats were trained in the spatial discrimination task (SD), which required a bar press opposite to the cue location for a reward (Fig. 8A). Each trial began with a 2-sec interval prior to the onset of one of two cues (yellow or red) at one of two locations (left or right). A correct response produced a food pellet (Noyes, 45 mg). An incorrect response produced a brief tone (95 dB, 500 ms) and terminated the trial, followed by a 2.5 sec penalty. A premature response prior to cue onset reset the trial. No response within two seconds from the cue onset (limited hold) terminated the trial. The inter-trial interval (ITI) was 10 sec. A training session ended after 100 pellets were delivered or 60 min elapsed. Once the animals' performance reached the behavioral criterion ($\geq 85\%$ correct, 3 consecutive sessions), reversal training began. Some rats switched to reversal 3–5 sessions earlier than the rest to avoid overtraining. The reversal task (SDR) required a bar press on the same side as the cue location (Fig. 8B). Except the requirement for correct responses, all task parameters in SDR were identical to those in SD. Rats ($n=34$) were trained up to 22 sessions on SDR. In both SD and SDR, percent correct responses (%CR), response latencies (correct and incorrect), the number of barpresses during the ITI, and the number of days to reach criterion ($\geq 85\%$ CR, 3 consecutive sessions) were measured. For the METH group ($n=17$), drug- and vehicle-treated rats were randomly assigned to eight operant chambers. For the PCP ($n=17$) group, the chambers for drug- and vehicle-treated rats were counterbalanced.

4.3. Data Analysis

The effects of METH and PCP on locomotor activity and social interaction were analyzed by two-way ANOVAs (treatments $\times$ sessions). LSD post-hoc was used for pair-wise comparisons. Activity of the control rats in METH and PCP groups did not differ during either the acute drug state or following drug treatment ($p>.05$), and were combined in data analysis. In SD and SDR, the percent correct responses (%CR), response latencies (correct and incorrect), and the number of bar presses during the ITI were analyzed by two-way ANOVAs. For rats that reached criterion earlier than the rest, missing values for %CR, latencies, and ITI barpresses were replaced with the value of their last session for statistical analysis. One-way ANOVA was used to compare the effects of drug on days to reach criterion. LSD post hoc analysis was used for pair-wise comparisons.

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Fig. 1.

Acute effects of METH and PCP on locomotor activity. Overall effects of acute METH (n=16) and PCP (n=16) on locomotion were measured after the first and third injections (**A**). The x-axis represents the order of injection. The y-axis represents the distance traveled (cm). Acute drug effects on locomotion were analyzed at 5-min intervals, across a 60-min period (**B** & **C**). The first METH injection increased locomotion during the first 25 min, followed by a decrease, whereas the first PCP injection gradually increased locomotion during the last 25 min (**B**). The third METH injection did not affect locomotion, whereas PCP further enhanced locomotion (**C**). In this and other figures, asterisks (*) denote $p < .05$ and error bars indicate standard error of the mean (SEM).

Fig. 2.

Locomotor activity following METH and PCP. All groups showed a gradual decrease in activity during the test sessions throughout post-drug periods: Day 3 (**A**), Day 7 (**B**), Day 14 (**C**), and Day 28 (**D**). Neither METH (n=16) nor PCP (n=16) significantly affected locomotion during the post-drug period. On Day 3, the METH group showed a decrease in locomotion during the first 5 min and between 20–25 min, yielding a significant interaction (treatment x time).

Fig. 3.

Social interaction following METH and PCP. Control rats showed a gradual increase in social interaction on Days 3–14, whereas the METH group ($n=28$, 4 rats/squad, 7 squads) showed a gradual decrease relative to controls on Day 7 and Day 14 (**A**), yielding a significant difference on Day 14 ($p<0.05$). METH ($n=28$, 4 rats/squad, 7 squads) did not affect social interaction on Day 28. Overall social interaction of the PCP group did not differ from that of controls on Days 7, 14, and 28 (Fig 3B, Top). The PCP group decreased social interaction during the first 8 min of the session (**B, Bottom**). The x-axis represents days after the last injection, and the y-axis represents total score of social interaction. Rats were coded by color (red=METH; black=saline; green and yellow=no treatment) and were tested in an open-field for social interaction (**C**). Examples of four METH groups were collected 7 and 14 days after the last drug injection. Rats in circles are METH-treated.

Fig. 4.

Effects of METH on spatial discrimination in adulthood. METH (n=8) and control groups (n=9) showed comparable performance during acquisition of spatial discrimination (**A**), yielding a similar number of training days to reach a criterion, 85% or greater percent correct for three consecutive sessions (**A; Inset**). Both groups showed a similar response latency, with a stable correct response latency across training sessions (**B, Top**) and a gradual decrease in incorrect response latency (**B, Bottom**). METH and control groups decreased number of barpresses during the intertrial interval (ITI) in a similar manner across the training sessions (**C**).

Fig. 5. Effects of PCP on spatial discrimination in adulthood. PCP (n=8) and control groups (n=9) showed comparable acquisition of spatial discrimination (**A**). Number of training days to reach the behavioral criterion did not differ between the two groups (**A; Inset**). Latencies of correct and incorrect responses (**B**) and ITI barpresses (**C**) were similar across the training sessions.

Fig. 6. Effects of METH on reversal learning in adulthood. Although there was no significant difference, the METH group ($n=8$) showed a slower trend in acquisition of reversal, compared to controls ($n=9$) (**A**), and required more training days to reach a criterion (**A, Inset**). Asterisks (*) denote $p < .05$ in pair-wise comparisons. Both groups showed a similar pattern of response latency (correct and incorrect) across the training sessions (**B**) and the number of barpresses during the ITI (**C**)

Fig. 7.

Effects of PCP on reversal learning in adulthood. The PCP group ($n=8$) showed a slower acquisition of reversal compared to controls ($n=9$) (A), requiring more training days to reach the behavioral criterion than controls (A, Inset). Both PCP and control groups showed a stable pattern of correct response latency across the training sessions (B, Top), and a similar decrease in incorrect response latency across the training sessions (B, Bottom). During the early phase of reversal (first 6 sessions), the PCP group showed a greater number of ITI barpresses, compared to controls (C).

Fig. 8.

The spatial discrimination task (SD) and the spatial reversal task (SDR). In the spatial discrimination task (**A**), a stimulus was presented either at right or left with two different colors (red or yellow) on each trial. A correct barpress opposite to the cue location was required to receive a food pellet. In the reversal task (**B**), a barpress near the same location as the cue was required to receive a pellet. All parameters were identical to those used in SD. Arrows indicate correct responses in different trial types during SD and SDR.