Short communication

The effects of phencyclidine pretreatment on amphetamine-induced behavior and c-Fos expression in the rat

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Abstract

Previous data demonstrate that a single injection of phencyclidine enhances amphetamine-induced behaviors 24 h later, suggesting that the delayed effects of a single dose of phencyclidine may produce a schizophrenia-like state in animals. These behavioral changes were accompanied by altered patterns of c-Fos induction, suggesting possible neurochemical correlates to the observed behaviors. Because investigations into PCP’s ability to model schizophrenia have found that the effects of repeated, or subchronic, PCP administration differ according to the dose and administration paradigm, this study sought to determine whether single and subchronic PCP exposure produce different effects on amphetamine-induced behaviors and c-Fos induction. No differences were observed between these administration paradigms; both single and subchronic PCP exposure enhanced amphetamine-induced c-Fos in the striatum, decreased c-Fos in the prefrontal cortex, and decreased the number of cage-crossings. However, the observation that PCP pretreatment affected c-Fos induction in the same manner observed previously while having an opposite effect on amphetamine-induced behavior suggests that these behavioral and neurochemical effects are dissociated.

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Phencyclidine (PCP) produces a schizophrenia-like state in humans [2,7] and has thus been repeatedly investigated for its ability to model schizophrenia-like behaviors in animals [7–10,12–15]. The delayed effects of PCP, those seen after intoxication has subsided, have been reported to induce a variety of schizophrenia-like behaviors [8–10,12–15] including enhanced behavioral responsiveness to amphetamine [9,15], thought to be an appropriate animal model for schizophrenia based on the observation that human schizophrenics demonstrate enhanced sensitivity to amphetamine [1]. Following exposure to a single high dose of PCP, this enhanced responsiveness to amphetamine is accompanied by increased striatal c-Fos and decreased basal c-Fos induction in the anterior cingulate cortex, suggesting possible neurochemical correlates to the behavioral change [15]. However, previous studies investigating the use of PCP to model schizophrenia have focused on repeated rather than single injections of PCP [8–10,13] and studies using different doses and schedules of administration have yielded disparate behavioral and neurochemical results [5,9,10,15]. Thus this experiment sought to determine whether single and subchronic PCP exposure have different effects on subsequent amphetamine-induced behavior and c-Fos expression.

Thirty-six male Sprague–Dawley rats (Charles River, Wilmington, MA) weighing 225–400 g were housed individually with access to food and water ad lib and maintained on a 12 h reverse light–dark cycle. Animals were acclimated for 60 min to the behavioral testing cage (a 12.5×24×12 in. glass aquarium with wood shavings located in a room illuminated with red light) once a day for 4 days. One hour following the end of each acclimation period, animals were injected with either PCP (15 mg/kg)
or vehicle (saline) such that three pretreatment groups were generated: vehicle (V; vehicle injections on Days 1–4), single PCP (PCP; vehicle on Days 1–3 and PCP on Day 4), and subchronic PCP (scPCP; PCP on Days 1–4). On the fifth day, animals were acclimated to the testing cage for 60 min and then injected with amphetamine (A; 0.5 mg/kg) or vehicle (V; saline) such that six treatment groups were generated: V–V, V–A, PCP–V, V–A, scPCP–V, and scPCP–A. The doses of PCP and amphetamine were chosen based on previous evidence that 15 mg/kg PCP alters the behavioral and neurochemical response to 0.5 mg/kg amphetamine [15]. Each group began with six rats but due to experimental error, behavioral data from one rat each was lost in the V–V, PCP–V, and scPCP–A groups and c-Fos data was lost for one rat in the V–V group. All injections were administered i.p. in 2 ml/kg.

Behavior was videotaped for 110 min following amphetamine or vehicle injection and locomotion and rearing were analyzed as described previously [15]. In addition, the number of seconds engaged in repetitive sniffing behavior was recorded in the amphetamine challenged rats during three two min periods (20–22, 40–42, and 60–62 min. following injection) and combined. 2 h following the injection, animals were anesthetized, perfused, and their brains processed for c-Fos immunohistochemistry according to previously published methods [15]. The number of immunoreactive cells were counted in the regions depicted in Fig. 1a by an experimenter blind to the treatment group. Behavioral measures and regional c-Fos counts were analyzed with a 2×3 ANOVA with the main factors of pretreatment (V, PCP, or scPCP) and challenge (V or A). Individual group differences were then assessed with one-way ANOVAs followed by Student–Newman–Keuls post-hoc tests. Sniffing in the amphetamine-challenged groups was compared with a one-way ANOVA.

Amphetamine challenge increased the number of FLI-positive cells in the striatum (F(1,29)=29.5, P<0.001) and there was a significant interaction between pretreatment and challenge (F(2,29)=4.2, P<0.05; Fig. 1b). A one-way ANOVA revealed a significant effect of group on the number of FLI-positive striatal cells (F(5,29)=6.3, P<0.001). Post-hoc tests revealed significant differences between V–A versus PCP–A, PCP–V versus PCP–A, and scPCP–V versus scPCP–A. The number of FLI-positive cells in the nucleus accumbens and anterior cingulate cortex was not altered by our treatments (Fig. 1c–e). Amphetamine challenge did not alter the number of FLI-positive cells in the PFC; however PCP pretreatment did significantly decrease PFC FLI (F(2,29)=4.5, P<0.05; Fig. 1f).

Amphetamine challenge increased the number of cage-crossings (F(1,27)=6.2, P<0.05) and rears (F(1,27)=6.3, P<0.05; Fig. 2). PCP pretreatment significantly decreased cage-crossing (F(2,27)=4.6, P0.05), but not rearing. There were no significant differences in the number of seconds of repetitive sniffing in the amphetamine-challenged groups (V–A=90±8, V–PCP–A=104±10, scPCP–A=75±12, F(2,15)=1.9).

These data demonstrate a similar pattern of amphetamine-induced behavior and c-Fos induction following single and subchronic high-dose PCP exposure. While most of the significant results indicate overall effects of PCP pretreatment without specific intergroup differences emerging from post-hoc tests, the effects seen in the single and subchronic PCP pretreatment groups are clearly in the same direction.

Amphetamine challenge enhanced both the number of cage-crossings and rears in accordance with previous studies demonstrating the psychomotor effects of amphetamine [3,5,6,9,11]. PCP pretreatment significantly decreased the number of cage-crossings but did not affect the number of rears, contrasting with our previous observation that pretreatment with a single injection of PCP enhances amphetamine-induced cage-crossings and rears in a similar paradigm [15]. This decrease does not appear to be due to an increase in stereotypies as there was no difference in the number of seconds engaged in sniffing behavior of the V–A, PCP–A, and scPCP–A rats. Previous studies have found that PCP pretreatment either increases [9] or decreases [5] subsequent responsiveness to amphetamine, depending on the dose and administration paradigm. In fact, Jentsch et al. have shown that rats have different neurochemical responses to the same dose of PCP administered on different schedules [10]. Thus both the behavioral and neurochemical effects of PCP pretreatment appear to be dependent on the dose and administration schedule.

While the behavioral effects of PCP pretreatment observed in this study are opposite those seen in the prior study, the effects on c-Fos are similar. The current observation that PCP pretreatment increases amphetamine-induced striatal FLI but decreases cage-crossings contrasts with our previous finding that PCP pretreatment increases both amphetamine-induced striatal FLI and cage-crossings. Taken together, the data from these two studies indicate that the effects of PCP pretreatment on amphetamine-induced locomotion and striatal FLI are dissociated. This dissociation is consistent with lesion studies which demonstrate that amphetamine-induced locomotion depends on the mesolimbic rather than the nigrostriatal dopamine system [3,4,11]. Despite this association between mesolimbic dopamine activity and locomotion, neither this study nor our previous study found changes in nucleus accumbens FLI accompanying changes in locomotion [15]. Thus this measure of mesolimbic activity may not reflect the neurochemical changes associated with locomotion.

The observation that subchronic PCP decreases dopamine turnover in the prefrontal cortex (PFC) has led to the suggestion that PCP may alter responsiveness to amphetamine via changes in PFC input to subcortical dopamine systems [10]. In our previous study, we argued that the presence of PCP pretreatment-induced decreases in FLI in the anterior cingulate cortex (AC) was consistent with the
Fig. 1. The areas of FLI quantification are indicated in (a). The area in which FLI was counted was 0.84 mm$^2$ in the striatum (S), 0.315 mm$^2$ in the nucleus accumbens core (Nc), 0.21 mm$^2$ in the nucleus accumbens shell (Ns), and 0.42 mm$^2$ in the anterior cingulate cortex (AC) and the prefrontal cortex (PFC). Amphetamine enhanced the number of FLI positive cells in the striatum following both single (PCP) and subchronic PCP (scPCP) pretreatment and the number of amphetamine-induced FLI positive cells was enhanced by single PCP pretreatment (b). The number of FLI-positive cells in the nucleus accumbens core (c), nucleus accumbens shell (d), and anterior cingulate cortex (e) were not affected by treatment group; however, PCP pretreatment induced an overall decrease in the number of FLI-positive cells in the prefrontal cortex (PFC). Six half brain sections were averaged for each region of each brain. Data are presented as mean of one half brain section ± SEM. * $P<0.05$, Student–Newman–Keuls post-hoc test for differences between treatment groups.
More importantly, the current data show that previously observed behavioral and neurochemical changes seen following PCP pretreatment are dissociated.

References