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Amphetamine disruption of prepulse inhibition of acoustic startle is reversed by depletion of mesolimbic dopamine

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Abstract. Previous studies have demonstrated that dopamine (DA) agonists disrupt sensorimotor gating as measured by prepulse inhibition (PPI) of the acoustic startle response (ASR) in rats; other reports suggest that this stimulant-induced disruption of PPI may reflect drug-induced increases in ASR amplitude rather than changes in sensorimotor gating. In the current study, 6-hydroxydopamine lesions that depleted dopamine from the nucleus accumbens, olfactory tubercles and anterior striatum reversed the disruption of PPI caused by amphetamine (AMPH), but did not disrupt AMPH potentiation of ASR baseline. These findings strongly suggest that increased mesolimbic DA activity is one substrate of the AMPH-induced disruption of PPI; in contrast, AMPH potentiation of baseline startle amplitude may be independent of mesolimbic DA activation.

Key words: Acoustic startle response – Dopamine – Nucleus accumbens – Prepulse inhibition – Schizophrenia

The startle reaction to a strong sensory stimulus is reduced by the presentation of a weak lead stimulus (Graham 1975). This reduction, termed "prepulse inhibition" (PPI), has been used as a measure of sensorimotor gating and is significantly diminished in schizophrenic patients (Braff et al. 1978). Recent studies in rats have shown that PPI is disrupted by systemic treatment with the dopamine (DA) agonists apomorphine or amphetamine (AMPH) (Mansbach et al. 1988) as well as the psychotogen phencyclidine and the NMDA antagonist MK801 (Geyer and Mansbach 1989). Apomorphine-induced disruption of PPI is reversed by systemic treatment with the DA receptor antagonist haloperidol (Mansbach et al. 1988), which has clinical antipsychotic activity.

While activation of brain DA systems may be a substrate for the loss of sensory gating that follows peripheral administration of DA agonists in rats, it is not known which brain DA systems are involved in this pharmacological effect. The mesolimbic DA terminal fields that include the nucleus accumbens (NAC) may be a critical region for the effects of DA agonists on PPI, since "subthreshold" doses of apomorphine disrupt PPI in rats with "supersensitive" NAC DA receptors following inta-NAC infusion of 6OHDA (Swerdlow et al. 1986a). If the loss of PPI caused by DA agonists results from increased mesolimbic DA activity, then interventions that block this increased DA activity should prevent the drug-induced changes in PPI. To test this, we measured ASR amplitude and PPI in rats treated with the indirect DA agonist AMPH (0 or 2.0 mg/kg SC) after sham- or 6OHDA-lesions of the mesolimbic DA system.

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Methods

Sixteen male albino Sprague-Dawley rats (225–250 g) were housed in pairs, and maintained on a reversed 12 h:12 h light/dark schedule (lights off at 0700 hours) with food and water provided ad libitum. Testing occurred between 0900 and 1300 hours.

One week after arrival, rats were anesthetized with pentobarbital (50 mg/kg), placed in a Kopf stereotaxic instrument with the toothbar 5 mm above the interaural line, and infused bilaterally with either vehicle (2 µl of 0.1% ascorbic acid in saline; "SHAM" group, $N=8$) or 6-OHDA (8 µg/2 µl, as salt; "6OHDA" group, $N=8$) through 30 ga cannulae aimed at the nucleus accumbens (AP+3.2 from Bregma, L +/−1.7, DV −7.8 from skull). Infusion rate was 1 µl/3 min, and cannulae were left in place for 1 min following infusion.

One week after surgery, one half of each group of rats was injected with either *d*-amphetamine sulfate (2.0 mg/kg SC) or saline vehicle (1 ml/kg SC). This dose of AMPH was previously demonstrated to disrupt PPI (Mansbach et al. 1988). Immediately following these injections, rats were placed in an acoustic startle chamber for a 5-min acclimation period with a 70 dB [A] background noise. The chambers (SR-LAB, San Diego Instruments, San Diego, CA) consist of a Plexiglas cylinder surrounded by and attached to a Plexiglas frame. The frame is suspended inside a rigid Plexiglas structure via four rubber cylinders, and the entire assembly is located within a ventilated enclosure. Acoustic tones are presented by a loudspeaker mounted 24 cm above the rat. A piezoelectric cartridge resting on the Plexiglas frame detects and transduces movement within the cylinder. Stabilimeter readings are rectified and recorded by a microcomputer and interface assembly (San

Diego Instruments), with 100 1-ms readings collected starting at tone onset. ASR amplitude was defined as the average of these readings.

Following the 5 min-acclimation period, rats experienced stimuli consisting of a startle pulse ("P": a 118 dB [A] 40 ms broad band burst) or a prepulse tone ("PP": an 80 dB [A] 20 ms broad band burst presented 100 ms prior to the onset of the startle tone). The test session included a series of four trial types: pulse alone (P-ALONE), prepulse followed by pulse (PP-P), prepulse alone (PP-ALONE) and no stimulus (NOSTIM). An initial P-ALONE trial was followed by 15 sequences of the four trial types, varied in order, for a total of 61 trials. Intertrial intervals averaged 15 s. The session was divided into two blocks of 31 and 30 trials for analysis of time course effects.

One week after the first test session, drug treatments were reversed, and rats were then returned to the stabilimeter cages and exposed to an identical test session. In this manner, each rat received both vehicle and AMPH, with the order of drug treatment (week 1 or 2) balanced between the two groups.

Following behavioral testing, all rats were decapitated, and their brains were removed. Free-hand dissection liberated the olfactory tubercles, NAC, anterior and posterior striatum, which were stored at −40° C until assayed for levels of DA and DOPAC using HPLC. Behavioral data was analyzed in two ways: P-ALONE amplitude was analyzed using a two-way ANOVA with repeated measures on drug treatment and time; prepulse inhibition, defined as ([PP-P÷P-ALONE]×100), was analyzed using a two-way ANOVA with repeated measures on drug treatment and time, with arcsin transformation performed to correct for percentage calculations. Level of significance was $P<0.05$.

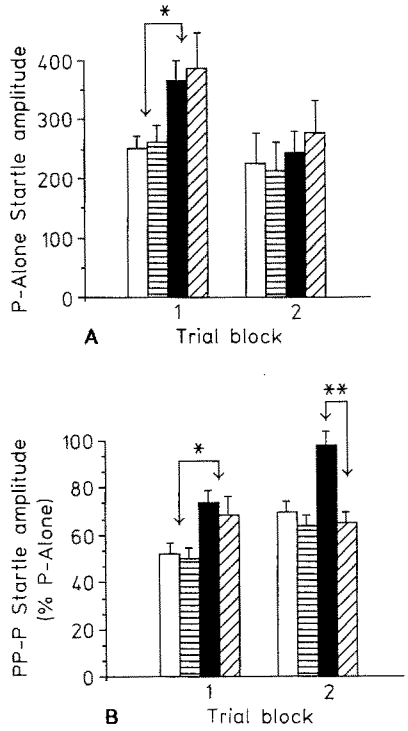

Fig. 1. **A** Effects of AMPH on baseline startle amplitude. Treatment (saline vs AMPH) served as a within-subject factor, while lesion (SHAM vs 6OHDA) served as a between-group factor. Startle amplitude of P-ALONE trials is the dependent variable. * Indicates significant main effect of drug ($P<0.05$) by ANOVA. **B** Effects of AMPH on prepulse inhibition of acoustic startle. Treatment (saline vs AMPH) served as a within-subject factor, while lesion (SHAM vs 6OHDA) served as a between-group factor. Per cent scores ([PP-P÷P-ALONE]×100) served as the dependent variable. * Indicates significant main effect of drug ($P<0.05$) by post-hoc ANOVA following significant treatment × time interaction. ** Indicates significant difference ($P<0.05$) by paired t-test following significant drug × lesion interaction. □ sham/saline; ▤ 6OHDA/saline; ■ sham/amph; ▨ 6OHDA/amph

Results

Regional biochemistry is shown in Table 1. Injections of 6OHDA resulted in significant depletion of DA from mesolimbic DA regions including the olfactory tubercles (76.9%; $t=4.95$, df 14, $P<0.001$), NAC (80.5%; $t=10.87$, df 14, $P<0.0001$) and anterior striatum (66.3%; $t=3.08$, df 14, $P<0.01$); there was also a small but significant depletion from the posterior striatum (15.5%; $t=2.34$, df 14, $P<0.04$). Significant depletion of DOPAC was noted in the NAC (84.5%) and anterior striatum (69.5%).

Drug and lesion effects on P-ALONE amplitude are seen in Fig. 1A. Two-way ANOVA with repeated measures on drug treatment and time revealed a near-significant effect of drug ($F=4.08$, df 1,14, $P=0.06$), no significant effect of lesion ($F=0.008$), or drug × lesion interaction ($F=0.3$). There was a significant effect of time ($F=22.38$, df 1,14, $P<0.0005$), but no interaction of time × lesion ($F=0.01$). There was no significant drug × lesion × time interaction ($F=0.1$), but there was a significant interaction of time × drug ($F=4.70$, df 1,14, $P<0.05$), and ANOVA revealed

Table 1. Dopamine and DOPAC levels in four brain regions following sham- or 6OHDA-lesions of the nucleus accumbens, expressed as ng/mg protein

	Regional neurochemistry							
	Olfactory tubercles		Nucleus accumbens		Anterior striatum		Posterior striatum	
	DOPAC	DA	DOPAC	DA	DOPAC	DA	DOPAC	DA
NAC sham	65.97 ±8.69	108.17 ±14.44	48.36 ±2.94	69.67 ±4.40	107.49 ±5.69	118.07 ±18.06	75.90 ±3.65	193.28 ±6.81
NAC 6OHDA	44.52 ±6.53	24.02 ±7.47	7.52 ±1.39	13.62 ±2.21	32.75 ±5.99	39.78 ±17.69	54.55 ±2.71	163.25 ±11.33
% depletion	32.5	76.9	84.5	80.5	69.5	66.3	28.1	15.5

a significant effect of drug during block 1 ($F=6.40$, df 1,14, $P<0.025$) with no drug × lesion interaction during block 1 ($F=0.08$). ANOVA for block 2 revealed no significant effect of drug or drug × lesion interaction ($F<1$ both comparisons). Thus, AMPH potentiated P-ALONE amplitude over block 1; this effect was lost during block 2. While these effects of AMPH on P-ALONE amplitude are short-lived compared to reports using much higher doses of AMPH (Kehne and Sorenson 1978), studies using comparable doses of AMPH report either no (Mansbach et al. 1988) or very slight (Swerdlow et al. 1986b) increases in P-ALONE amplitude. Neither baseline ASR (saline) or AMPH-potentiated ASR were significantly changed by mesolimbic DA depletion.

Drug and lesion effects on PPI are seen in Fig. 1 B. Two-way ANOVA with repeated measures on drug treatment and time revealed a significant effect of drug ($F=14.38$, df 1,14, $P<0.005$), a significant main effect of lesion ($F=4.59$, df 1,14, $P=0.05$), and a significant effect of time ($F=20.02$, df 1,14, $P<0.0005$). There was a significant drug × time interaction ($F=7.33$, df 1,14, $P<0.02$), but no other significant two- or three-way interactions ($F<1$ all comparisons). ANOVA for block 1 revealed a significant effect of drug ($F=10.16$, df 1,14, $P<0.007$), but no effect of lesion or lesion × drug interaction ($F<1$ both comparisons). ANOVA for block 2 revealed a significant effect of drug ($F=6.74$, df 1,14, $P<0.025$), a significant effect of lesion ($F=9.82$, df 1,14, $P<0.01$), and a significant lesion × drug interaction ($F=5.25$, df 1,14, $P<0.04$). Individual paired t-tests revealed that AMPH decreased PPI in SHAM group animals ($t=2.83$, df 7, $P<0.025$), but not in 6OHDA-lesioned animals ($t=0.30$). Thus, AMPH caused a decrease in PPI in SHAM group animals, but this effect was prevented in block 2 by 6OHDA lesions. Independent analysis revealed that there was no effect of drug order, i.e. the lesion effect was equally robust for animals that received AMPH during the 1st week as it was for animals that received AMPH during the 2nd week (mean % P-ALONE values for AMPH-treated SHAM versus 6OHDA-animals week 1: 95.00% versus 66.25%, respectively; week 2: 99.52% versus 63.95%, respectively). Time course analysis revealed that PPI was inhibited by AMPH to a greater degree in block 2 than in block 1 ($t=3.08$, df 7, $P<0.02$), while P-ALONE amplitude was potentiated by AMPH to a greater degree in block 1 than in block 2 ($t=3.64$, df 7, $P<0.01$). Thus, the effects of AMPH on ASR baseline and on PPI are dissociable by lesion effects and by time course.

Discussion

These results suggest that deficits in PPI following AMPH treatment result at least in part from AMPH-induced increases in mesolimbic DA activity. As previously reported (Mansbach et al. 1988), AMPH treatment caused a disruption of PPI in animals with intact mesolimbic DA activity. In the current study, this effect of AMPH on PPI was reversed in animals that had sustained depletion of mesolimbic DA by prior 6OHDA infusion into the NAC. These results have several implications.

First, these findings support previous work (Swerdlow et al. 1986a) implicating mesolimbic DA overactivity as a substrate for disruption of PPI in rats. The neural substrates responsible for a loss of PPI may be relevant to clinical psychiatry, since sensorimotor gating deficits occur in patients with schizophrenia (Braff et al. 1978). Thus, like rats treated with AMPH (Mansbach et al. 1988; present findings), schizophrenic patients exhibit abnormally low levels of PPI (Braff et al. 1978). Extrapolation from our preclinical findings suggests that mesolimbic DA hyperactivity might account for deficiencies of PPI in schizophrenia; such a hypothesis is consistent with evidence of mesolimbic DA hyperactivity in the pathophysiology of this illness (Crow et al. 1984; Wong et al. 1986).

Second, our results address a recent failure to replicate the finding of Mansbach et al. (1988) that DA agonists disrupt PPI in rats. In this work by Davis (1988), neither apomorphine nor AMPH blocked PPI in rats. Subsequent studies in both of these laboratories (Davis et al. in preparation), revealed that the differences between their findings resulted from differences in the choice of background white noise (30 dB [A] :(Davis 1988) versus 70 dB [A]: Mansbach et al. 1988). This clarification suggests that the loss of PPI following treatments with DA agonists is not simply a reflection of stimulant-induced increases in ASR baseline. Our present results indicate that the effects of AMPH on ASR baseline and on PPI are in fact anatomically dissociable, since AMPH potentiation of ASR baseline is not reduced by mesolimbic DA depletion, while AMPH disruption of PPI is reversed by these lesions. These effects of AMPH appear to be temporally dissociable as well: in our test session, the effects of AMPH on baseline ASR are significantly greater in the first trial block than in the second, while the effects of AMPH on PPI are significantly greater in the second trial block than in the first. In summary, AMPH-induced disruption of PPI is reversed following depletion of DA from the NAC, olfactory tubercles and anterior striatum; AMPH potentiation of baseline startle amplitude is not significantly effected by NAC 6OHDA infusions. Mesolimbic modulation of AMPH-induced changes in sensorimotor gating may have implications for the normal regulation of appetitive behaviors as well as the disruption of sensorimotor gating in states of mesolimbic DA overactivity that may accompany schizophrenia.

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