Prepulses Inhibit Startle-Induced Reductions of Extracellular Dopamine in the Nucleus Accumbens of Rat

Trevor Humby,¹ Lawrence S. Wilkinson,¹ Trevor W. Robbins,¹ and Mark A. Geyer²

¹Department of Experimental Psychology, University of Cambridge, Cambridge CB2 3EB, United Kingdom, and

²Department of Psychiatry, University of California at San Diego, La Jolla, California 92093

In vivo brain microdialysis was used to monitor extracellular levels of dopamine (DA) in the nucleus accumbens (NAc) of rats during exposure to startling acoustic stimuli. Ten rats were prepared with guide cannulae into which dialysis probes were inserted 1 d before testing. Two to three hours after the start of perfusion, rats were placed into the startle chamber and exposed to a continuous 70 dB(A) background noise. Dialysis samples (2.0 μ l/min) were collected at 6 min intervals. Startle pulses (120 dB[A] noise) were presented in 20-trial blocks lasting 5 min. In some blocks, an 86 dB[A] prepulse preceded each of the 20 pulses by 100 msec, with the order of presentation of pulse-alone or prepulse + pulse blocks being counterbalanced between animals. Three to six sample periods intervened between stimulus-containing blocks. Monoamine

and metabolite levels were measured using HPLC with electrochemical detection. During the presentations of startling stimuli, DA levels in the NAc decreased relative to the immediately preceding 12 min baseline. This decrease in DA was maintained for only one additional sample period. By contrast, the presentation of prepulse + pulse trials failed to affect dialysate levels of NAc DA during or immediately after the stimulation. Thus, startling acoustic stimuli produce significant and transient decreases in dialysate levels of DA in the NAc. Furthermore, prepulse stimuli effectively inhibit these neurochemical effects of startling stimuli, in parallel with their established ability to inhibit the amplitude of the behavioral startle response.

Key words: prepulse inhibition; startle; dopamine; schizophrenia; microdialysis; nucleus accumbens

The startle response is an unconditioned response to a sudden, intense sensory stimulus that has been used to study the neural mechanisms underlying several forms of behavioral plasticity. For example, when a startle-eliciting stimulus is preceded by a weak lead stimulus, the amplitude of the startle response is decreased markedly (Hoffman and Ison, 1980; Ison and Hoffman, 1983). This cross-species phenomenon, called prepulse inhibition, provides an example of sensorimotor gating mechanisms that presumably act to buffer or filter exteroceptive stimuli (Braff and Geyer, 1990). Schizophrenic patients and nonmedicated schizotypal subjects exhibit less prepulse inhibition than do normal subjects (Braff et al., 1978, 1992; Cadenhead et al., 1993; Bolino et al., 1994).

Efforts to develop animal models of the gating deficits in schizophrenia have focused on manipulations that activate central dopamine (DA) systems (Geyer et al., 1990). In rats, prepulse inhibition is decreased consistently by manipulations that increase the activity of mesolimbic DA systems. For example, prepulse inhibition is disrupted by systemic administration of indirect DA agonists such as amphetamine (Mansbach et al., 1988) or direct D2, but not D1, agonists (Peng et al., 1990). Furthermore, the effects of apomorphine on prepulse inhibition are blocked by D2 antagonists (Swerdlow et al., 1991) and appear to be attributable

to DA overactivity specifically within the mesolimbic DA system innervating the nucleus accumbens (NAc) (Geyer et al., 1990; Swerdlow et al., 1992). Accordingly, it has been suggested that the reduction in prepulse inhibition produced by DA agonists in rats may serve as an animal model of the qualitatively similar behavioral deficits in sensorimotor gating exhibited by schizophrenic patients (Geyer and Braff, 1987; Rigdon and Viik, 1991; Swerdlow et al., 1994).

Despite the large body of evidence indicating that manipulations of mesolimbic DA can modulate the expression of prepulse inhibition, there have been no attempts, to our knowledge, to monitor directly the dynamics of DA transmission coincident with this phenomenon. Hence, in the present work we used the techniques of *in vivo* brain microdialysis to determine whether reliable changes in microdialysate measures of NAc DA would be induced by presentations of startling acoustic stimuli and whether any such changes, if observed, would be altered by the antecedent presentation of inhibitory prepulse stimuli. To enable simultaneous measurements of dialysate DA and startle responses, the standard procedures for demonstrating prepulse inhibition of acoustic startle were modified according to the results of an initial experiment in unoperated rats. A priori, it is difficult to justify specific predictions about the possible pattern of DA release that might be anticipated here. Nevertheless, there are precedents indicating that exposure to brief sensory stimuli can induce transient changes in NAc DA transmission (Abercrombie et al., 1989; Phillips et al., 1993; Young et al., 1993; Wilkinson, 1994).

MATERIALS AND METHODS

Subjects. The subjects in San Diego were 16 Sprague–Dawley-derived male rats (Harlan, San Diego, CA) with an average weight of 322 ± 10 gm. These rats were housed in pairs on a reversed 12 hr light/dark cycle (lights off at 7:00 A.M.). The subjects in Cambridge were 10 Lister

Received Nov. 10, 1995; revised Dec. 18, 1995; accepted Dec. 20, 1995.

This work was supported by a Wellcome Trust Program Grant (T.W.R.) and by National Institute of Mental Health (NIMH) Grant R37MH42228. M.A.G. was supported by NIMH Research Scientist Award K05MH01223 and by a Fogarty Senior International Fellowship (National Institutes of Health Grant F6TW01813).

Correspondence should be addressed to Mark A. Geyer, Department of Psychiatry, 0804, University of California at San Diego, La Jolla, CA 92093.

Dr. Humby's present address: MRC Cambridge Centre for Brain Repair, University of Cambridge, Cambridge CB2 3EH, UK.

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Figure 1. Schematic showing the general arrangement of the dialysis apparatus relative to the startle apparatus and details of the fluid lines, cannula, and dialysis probe used.

hooded male rats (OLAC, Bicester, UK) with an average weight of 377 ± 7 gm. These subjects were housed in pairs on a 12 hr light/dark cycle (lights on at 7:00 A.M.). Subjects had free access to food and water at all times except during testing.

Behavioral apparatus. Startle testing was accomplished with SR-LAB (San Diego Instruments, San Diego, CA) test stations. Each illuminated, ventilated chamber contained a stabilimeter composed of an 8.2 cm diameter Plexiglas cylinder mounted on a Plexiglas base as described previously (Geyer et al., 1993; Wilkinson et al., 1994). For the microdialysis studies, the cylinder was adapted slightly by removing the top half of the cylinder and extending walls vertically to provide more clearance. The adjustable flat lid of this modified chamber was equipped with a 1-cm-wide slot that extended from the middle to the front of the longitudinal axis of the chamber, such that the rat could be introduced into and removed from the cylinder with the microdialysis tubing being attached (see Fig. 1). A speaker mounted 24 cm above the animal provided both the background noise and the startle and prepulse stimuli, which were controlled by the SR-LAB. Visual stimuli could be presented by means of two 2.5 W light bulbs mounted on one of the walls of the chamber. Startle responses were transduced by a piezoelectric transducer mounted below the cylinder, digitized (0–4095 arbitrary units), rectified, and recorded as 100 readings of 1 msec duration each, starting at the onset of each startle stimulus. The average of these 100 readings was used as the dependent measure of each startle response.

Microdialysis apparatus. A Harvard perfusion pump (Harvard, Edenbridge, Kent, UK) was situated on the top of the startle chamber. Fluid lines (2.5 ml gas-tight syringes/FEP tubing) ran into the chamber via a hole at the back of the apparatus to connect with a single-channel liquid swivel (Biotech, Kimpton, UK). The liquid swivel was held by a clamp 16 cm above the top of the Plexiglas cylinder and was connected in turn to a spring (Plastics One, Roanoke, VA), down which both inlet and outlet lines could be threaded ready for connection to the inlet and outlet ports of dialysis probes protruding from the head of the subject (see Fig. 1). The spring itself was attached to the subject's head at an anchor point made from a piece of threaded plastic rod affixed to the skull with dental acrylic. Dialysates were collected at a point 14 cm below the swivel. Dialysis probes were made of silica glass and were of concentric design with a 2 mm dialyzing surface (Hospal, 250 μ m diameter, nominal molecular weight cutoff 5000 Da).

Surgical procedure. Seven days before the beginning of the startle session, the subjects were cannulated under Sagatal (60 mg/kg) anesthesia. Cannulae were made from cut-down syringe needles (21 gauge) and were positioned so that, when the dialysis probe was inserted into the cannula, the tip of the probe was at the following coordinates taken from the atlas of Pellegrino et al. (1979): AP +3.4 mm from bregma; LAT ± 1.5 mm; VERT 7.5 mm from dura. Cannulae were placed on the left and right in alternate subjects. Anchor points (10 mm diameter threaded nylon pedestals) were placed in a central position ~ 6 mm behind bregma. Both the cannulae and the anchor points were held in place with dental acrylic and skull screws.

Behavioral procedure. To test the validity of using a blocked design for the demonstration of prepulse inhibition, an initial study was conducted in unoperated rats (Sprague-Dawley). In contrast to the typical design of prepulse inhibition experiments, in which pulse-alone and prepulse + pulse trials are intermixed on a pseudorandom schedule, the temporal resolution of the microdialysis technique that was used required that 5 min blocks of either all pulse-alone or all prepulse + pulse trials be presented, with at least a 5 min interval of only background noise intervening between blocks. The first experiment tested whether such a design would demonstrate robust prepulse inhibition and whether this prepulse inhibition was sensitive to the effects of the noncompetitive NMDA antagonist dizocilpine, which is known to disrupt prepulse inhibition when traditional test designs are used (Mansbach and Geyer, 1989). Two groups of eight rats each were tested after subcutaneous injections of saline or 0.5 mg/kg dizocilpine (MK-801, Merck, Darmstadt, Germany). Ten minutes after injection, rats were placed into the startle chambers for a 5 min acclimation period [with a 70 dB(A) background noise] followed by alternating blocks of 20 pulse-alone stimuli or 20 prepulse + pulse stimuli. Blocks were separated by 5 min periods of the background noise. Pulses were 30 msec bursts of broadband noise at 120 dB(A) (all sound levels refer to the A scale); prepulses were 30 msec bursts of noise at 8 dB above the background presented 100 msec before the pulses. Sound levels were measured as described previously (Mansbach et al., 1988). In the case of the dialysis studies, probes were implanted the day before testing, and the animals were left overnight in a holding cage in the test room. At the beginning of the test day (between 8:00 and 9:00 A.M., 15–18 hr after probe implantation), the spring was attached to the anchor point and the fluid lines connected to the inlet and

outlet tubes of the dialysis probe. Once flow was established, samples were taken every 20 min for 150 min, and then four 6 min baseline samples were collected in the holding cage. Subsequently, samples were taken at 6 min intervals. This sampling interval was chosen because preliminary studies had established that the dead space from the dialysis probe to the collection point was equivalent to a delay of 40 sec. Given that startle stimuli were presented in 5 min blocks, a sampling period of 6 min was required to ensure that the dialysis bin subsumed the stimulus bin. After the baseline sampling period, subjects were moved into the Plexiglas tube within the startle chamber and left for 30 min, during which a 70 dB background noise was administered. After acclimation, the subjects received two blocks of pulse-alone trials and two blocks of prepulse + pulse trials. A pulse-alone block consisted of 20 presentations of a 120 dB startle stimulus of 30 msec duration, spaced evenly over a period of 5 min. A prepulse + pulse block consisted of 20 presentations of the same startle stimulus preceded (100 msec) by a 16 dB (above background) prepulse stimulus of 30 msec duration. Again, the 20 presentations were spaced evenly over a 5 min bin. Blocks of pulse-alone and prepulse + pulse trials were presented in two orders (order 1: pulse, pulse, prepulse, prepulse; order 2: prepulse, prepulse, pulse, pulse). The interval between blocks of trials was typically 24 min (equivalent to 4 samples). At the end of the startle trials, six animals also received a single presentation of an aversive conditioned stimulus (CS') while still in the startle chamber. The CS' comprised a 30 sec presentation of a flashing light (2.5 W) that had been paired with a mild footshock reinforcer the previous day in a separate box, as described elsewhere (Wilkinson et al., 1995). Eight light-footshock (0.5 sec/0.5 mA) pairings were given over a 20 min period; the footshock overlapping with the last 0.5 sec of a 30 sec flashing light (2.5 Hz). Finally, all animals were removed from the startle chamber and placed back in the holding cage, where D-amphetamine sulfate (Sigma, Poole, UK) was administered (1.5 mg/kg, free base, i.p.) and samples taken for another 72 min.

Dialysis procedure. Implanted probes were perfused at 2 μ l/min with an artificial CSF composed of (in mM) NaCl 124, KCl 3, MgCl₂ 1, CaCl₂ 1.8, pH 6.7. Preliminary studies had established that at room temperature the *in vitro* recovery of the probes for DA was $15 \pm 2\%$. Samples were collected into cut-down plastic Eppendorf tubes containing 2 μ l of 0.1 M perchloric acid and taken immediately for analysis.

HPLC analysis of dialysate. Levels of DA, dihydroxyphenylacetic acid (DOPAC), and the serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) were determined using small-bore HPLC coupled to electrochemical detection. Ten microliter samples were injected into a reverse-phase analytical column (Spherisorb, C18 S50DS2, 2 mm inner diameter, Phase-Sep Ltd., Deeside, UK) perfused at a flow rate of 200 μ l/min with a mobile phase containing (in mM): citric acid 152, sodium acetate 15, octyl sulfate 1, EDTA 0.8, and 8% methanol, pH 3.6. The analytical column was connected to a glassy carbon electrode held at +0.7 V relative to a Ag/AgCl reference electrode by a BAS LC-4B controller. The output from the working electrode was integrated using Gynkosoftware (Severn Analytical, UK). Chromatographic peaks were identified/quantified by reference to known concentrations of standards. Under the conditions described, the maximum sensitivity for DA was 0.25 pg on column (S/N ratio of 3).

Histology. At the end of the test session, animals were anesthetized deeply by an overdose of Sagatal (i.p.) and perfused transcardially with PBS, pH 7.4, containing 1% sodium nitrite for 2 min, followed by 250 ml of 4% paraformaldehyde in PBS containing 4% picric acid. The brains were removed from the skull and post-fixed in the same solution for 1 hr, then transferred to 25% sucrose in Tris-buffered saline. After equilibration in the sucrose, sections were cut on a freezing-stage sledge microtome at a thickness of 60 μ m into Tris-buffered saline, pH 7.4, and stored at 4°C before staining. One in five series was mounted onto gelatin-coated slides and stained for cresyl violet. After staining, slides were dehydrated in alcohols, cleared in xylene, and coverslipped.

Data analysis and statistics. Data were analyzed using the BMDP statistical package (Dixon, 1990). The behavioral data were analyzed in mixed-design ANOVAs of the averaged startle responses for each block, with dose of dizocilpine as a between-subjects factor (when appropriate, DRUG) and both repetitions of blocks (BLOCK) and type of trial block (pulse-alone or prepulse + pulse, CONDITION) as repeated measures. Prepulse inhibition was also calculated for each subject as the percent decrease in startle between the pulse-alone and prepulse + pulse conditions and analyzed in a one-way ANOVA with dose of dizocilpine as a between-subjects factor. For microdialysis samples, the two 6 min samples immediately preceding an event were averaged to provide a baseline

measure. Analyses of variance and covariance (ANCOVA) on the absolute levels of dialysate DA were conducted with the baseline values as the covariates and the 6 min samples during and immediately after each event as the dependent variables. The two time-bins (BIN), repetitions of events (BLOCK), and type of event (pulse-alone or prepulse + pulse blocks, CONDITION) were treated as repeated-measures factors. A similar ANCOVA was conducted with order as an additional (between subjects) factor (ORDER). Confirmatory analyses were done using either difference scores or percent change scores as the dependent measures. When overall analyses were significant, simple main effects were analyzed with ANOVA.

RESULTS

Validation of blocked design for prepulse inhibition

The averaged startle responses for rats treated with either saline or dizocilpine are shown for both pulse-alone and prepulse + pulse blocks in Figure 2. The critical result of this study was that robust prepulse inhibition was observed in the control subjects, despite the use of the unusual blocked-trials design. Saline-treated rats exhibited $68.4 \pm 5.4\%$ inhibition of startle, as expected of 8 dB prepulse stimuli (cf. Geyer et al., 1993). By contrast, the dizocilpine-treated rats exhibited only $31.0 \pm 8.0\%$ prepulse inhibition, as expected after this dose of dizocilpine (cf. Mansbach and Geyer, 1989). This difference was confirmed by one-way ANOVA on DRUG ($F_{(1,14)} = 15.0, p < 0.002$). Furthermore, the observation of robust prepulse inhibition was supported in the three-way ANOVA as a significant main effect of CONDITION ($F_{(1,14)} = 77.0, p < 0.0001$) in the absence of any significant effect of BLOCK or interaction between BLOCK and CONDITION. Thus, the blocked-trials design was effective in demonstrating prepulse inhibition that was independent of the repetitions of blocks and was sensitive to an established pharmacological treatment.

Verification of dialysis probe sites

Figure 3 shows an example of the dialysis probe location using the coordinates detailed in the methods. All probes were placed within the medial NAc corresponding to what has been defined as the "shell" region of the ventral striatum (Heimer et al., 1991). The anteroposterior range of the probe sites was <1 mm.

Basal levels of dialysate DA, DOPAC, and 5-HIAA in holding cage and on transfer to startle chamber

Figure 4, A, B, and C, show, respectively, dialysate DA, DOPAC, and 5-HIAA levels as determined in the holding cage and on transfer to the startle chamber. Basal dialysate levels of all three substances were stable and within the range found by ourselves and others in the NAc (Wilkinson et al., 1993; Young et al., 1993). Transfer of the animals to the startle chamber was without effects on dialysate DA, DOPAC, or 5-HIAA.

Effects of startle testing

Startle responses

The averaged startle responses for pulse-alone and prepulse + pulse blocks are shown in Figure 5, A and B, respectively. As with the validation study, there was evidence of robust amounts of prepulse inhibition. This observation was confirmed statistically by a significant main effect of CONDITION ($F_{(1,9)} = 47.9, p < 0.0001$) in the two-way ANOVA. In addition, there was a significant main effect of BLOCK ($F_{(1,9)} = 20.3, p < 0.002$) and a significant CONDITION by BLOCK interaction ($F_{(1,9)} = 13.4, p < 0.01$), both reflecting the habituation of startle responses to the pulse-alone stimuli (compare Fig. 5). Further analyses confirmed that the 16 dB prepulse stimuli reduced startle responses by $65.8 \pm 7.1\%$.

Figure 2. The effects of dizocilpine (0.5 mg/kg, s.c.) on startle responses in unoperated rats under blocked, pulse-alone (120 dB), and blocked, prepulse (120 dB + 16 dB prepulse) conditions. Each block, consisting of 20 trials given over 5 min, was presented twice in an alternating order. Data are the mean $\pm$ SEM ($n = 8$ /group).

Changes in DA levels

Figure 6, *A* and *B*, illustrates the changes in dialysate DA levels on presentation of the pulse-alone and prepulse + pulse stimuli, respectively. The data are shown for individual animals and are expressed as the differences (in fmol) between the dialysate levels during and immediately after the blocks of startle stimuli and the mean value of the preceding two baseline samples. In the pulse-alone condition (Fig. 6*A*), there was evidence of decreases in DA levels during these sample periods (note that dialysate DA had returned to baseline levels by the next collection period, i.e., 7–12 min after the offset of the startle stimuli; data not shown). In contrast, DA levels remained essentially unchanged in the prepulse + pulse condition (Fig. 6*B*). ANCOVA of the absolute levels of dialysate DA from which the difference scores were derived, covarying with baseline DA and including factors CON-

DITION, BIN, and BLOCK, revealed a main effect of CONDITION ($F_{(1,8)} = 10.38$, $p < 0.02$). Thus, there was a significant difference in DA levels between the pulse-alone and prepulse + pulse conditions after taking baseline levels of DA into account. The only other significant effect was the CONDITION by BIN interaction ($F_{(1,8)} = 6.18$, $p < 0.05$), reflecting the slight reduction in the effect of the pulse-alone trials after relative to during the stimulus presentations (compare Fig. 6). There were also main effects of CONDITION in the ANOVAs of the difference ($F_{(1,8)} = 16.11$, $p < 0.004$) and percentage difference data ($F_{(1,8)} = 75.03$, $p < 0.0001$). In terms of mean percent changes, the decreases in DA levels in the pulse-alone condition ranged between -16% and -29% . A separate ANCOVA with the additional factor ORDER run on the absolute levels of DA, confirmed that the order in which the blocks of startle stimuli were presented had no effects on dialysate DA levels (ORDER, $F_{(1,7)} = 1.69$; not significant). Correlational analyses failed to reveal any significant correlations between dialysate DA levels and the behavioral measures of startle reactivity or prepulse inhibition, as might be expected from the limited sample size in the present study. Similarly, the six animals that received footshock the previous day did not differ appreciably from the other four animals on either dialysate or behavioral measures. There were no significant changes in dialysate DOPAC or 5-HIAA levels under any of the pulse-alone or pulse + prepulse conditions.

Effects of aversive CS on dialysate DA levels

The change in dialysate DA levels on presentation of the 30 sec aversive CS⁺ is shown in Figure 7. Again, the data are shown for individual animals and are expressed as the differences, in fmol, between the dialysate levels during and immediately after the CS⁺ and the mean value of the preceding two baseline samples. Dialysate DA was elevated during the sample bin when the CS⁺ was presented, returning to baseline in the next sample bin. ANCOVA of the absolute levels of dialysate DA from which the difference values were derived, covarying with baseline DA and including the factor BIN, revealed a main effect of BIN ($F_{(1,5)} = 12.22$, $p < 0.02$). Hence, there was a significant difference in DA levels

Figure 3. Photomicrograph (40 $\times$) showing a typical dialysis probe placement (arrow). The anterior–posterior level of the section approximates plate 14 of the atlas of Paxinos and Watson (1986), i.e., 1 mm anterior to bregma. The section was stained with cresyl violet. *aca*, Anterior commissure; *LV*, lateral ventricle; *ICjM*, islands of Calleja major; *LAT.*, lateral; *MED.*, medial.

Figure 4. Dialysate levels of DA, DOPAC, and 5HIAA, in 6 min sampling bins, determined in the holding cage and on placement into the startle apparatus (shown by the arrow). Data are the mean $\pm$ SEM ($n = 10$).

between the sample for which the CS⁺ was present and the subsequent sample, taking baseline levels of DA into account. There was also a significant main effect of BIN in the ANOVA of the percentage change scores ($F_{(1,5)} = 8.37$, $p < 0.05$). In percentage terms, the change in DA levels for individual animals on presentation of the CS⁺ ranged between -28 and $+121\%$.

Effects of systemic D-amphetamine on dialysate DA levels

Figure 8 illustrates the change in dialysate DA levels after administration of 1.5 mg/kg D-amphetamine. The data are the means for the differences, in fmol, between dialysate levels subsequent to the drug challenge and the mean of the two preceding baseline samples. D-Amphetamine increased dialysate DA, reaching a maximum 30–40 min after the injection. Because the mean base-

line level of DA before drug treatment was 17.12 ± 0.14 fmol/10 μ l sample, the maximum increase in dialysate DA was $\sim 650\%$. There was a significant main effect of BIN on absolute levels of DA after covarying for baseline levels of DA ($F_{(11,88)} = 3.11$, $p < 0.002$).

DISCUSSION

The main findings of the study were that startling acoustic stimuli produced a transient and significant decrease in dialysate levels of DA in the NAc and that presentation of prepulse stimuli before the startling stimuli effectively blocked this decrease. Simultaneous monitoring of the behavior of the animals demonstrated that these changes in dialysate DA were accompanied by normal levels of both startle reactivity and prepulse inhibition. Subsequently, in the same animals, increases in dialysate DA levels were observed both on presentation of an aversive CS⁺ and after the systemic administration of D-amphetamine, confirming that the startled animals remained appropriately responsive to other manipulations of the NAc dopaminergic system.

Influence of microdialysis methodology and blocked-trial design

The possibility that the general significance of the data were compromised by the particular methods used in combining *in vivo* microdialysis and startle techniques would seem unlikely for several reasons. First, preliminary experiments conducted on unoperated animals established that the blocked-trial design gave qualitatively similar results to the more usual pseudorandom single-trial design in terms of both prepulse inhibition and the disruptive effects of the NMDA antagonist dizocilpine. Second, animals that were being dialysed exhibited normal levels of startle reactivity and prepulse inhibition and did not appear to be agitated or stressed. Third, placement into the startle apparatus was without effects on levels of DA, DOPAC, or 5-HIAA. Fourth, the changes in dialysate DA levels occasioned by the startling stimuli were seen in animals displaying the expected responses to both physiological (aversive CS⁺; Young et al., 1993; Wilkinson, 1994) and pharmacological challenges (D-amphetamine; Kuczenski et al., 1991).

Implications for dopaminergic effects on manipulations of acoustic startle and acoustic startle per se

The main impetus for the present investigation was the extensive converging evidence implicating dopaminergic systems within the NAc in the modulation of prepulse inhibition in rats (Swerdlow et al., 1992). The potential importance of this dopaminergic modulation of prepulse inhibition has been suggested by the observation of deficits in this form of sensorimotor gating in schizophrenia-spectrum subjects (Braff et al., 1992; Bolino et al., 1994). The main finding here of changes in NAc DA induced by startling stimuli, and the apparent inhibition of these changes by prepulse stimuli, provides further converging evidence for the involvement of this system in the modulation of prepulse inhibition. Furthermore, the observation that DA levels were found to be elevated in the prepulse + pulse condition relative to the pulse-alone condition might have been anticipated in the light of the pharmacological evidence that activation of dopaminergic systems in the NAc (Peng et al., 1990; Wan et al., 1994) disrupts prepulse inhibition. Clearly, however, it is difficult to predict the precise direction of change in dialysate levels of a monoamine in association with a behavioral phenomenon on the basis of the influences of pharmacological agents on that behavior. What is most important in the present context is that there was a differ-

Figure 5. Startle responses in rats undergoing *in vivo* microdialysis. Each animal received two blocks of pulse-alone (120 dB) and two blocks of prepulse (120 dB + 16 dB) trials in a counterbalanced manner (see Materials and Methods). Note the difference in absolute levels of startle shown here compared to Figure 2 attributable to different gain settings of the stabilimeters. Data are the mean $\pm$ SEM ($n = 10$).

ence between the pulse-alone and the prepulse + pulse condition, regardless of the nature of that difference.

Although the present studies were predicated on the established involvement of mesolimbic dopaminergic systems in the inhibition of startle by prepulses, predictions regarding the potential responsiveness of NAc DA to the presentation of startling stimuli per se were less obvious. Nevertheless, the most striking finding of the present studies was that repeated presentations of startling stimuli over a 5 min period produced transient decreases in dialysate DA in the NAc. This decrease was evident during and for only 6 min after the period of stimulation. As with other such presentations of brief sensory stimuli (e.g., conditioned stimuli or repeated footshock), one cannot exclude the possibility that the limited temporal resolution provided by *in vivo* microdialysis obscured the detection of biphasic changes in the levels of extracellular DA. In the present context, it is possible that the initial startle stimulus produced a transient increase in DA release that was followed by a more prolonged decrease. Thus, the observed decrease over the 6 min sampling period used here could reflect the net integration of such a biphasic response. Other approaches (e.g., voltammetry) will be required to answer this question definitively.

Mechanism of startle-induced changes in NAc DA

The mechanisms underlying the startle-induced decreases in NAc DA remain to be determined. One possibility is that these intense

sensory stimuli inhibit the firing of the ventral tegmental neurons that provide the dopaminergic innervation of the NAc (Horvitz et al., 1993). However, it has been shown that other unconditioned, intense sensory stimuli, such as repeated electric tail/footshock, typically produce *increases* rather than *decreases* in microdialysis measures of NAc DA (see review by Salamone, 1994). Indeed, the only reports of stimulus events that produced decreased levels of NAc DA involved presentations of gustatory or olfactory stimuli signaling conditioned aversion responses (Mark et al., 1991; Louilot et al., 1992). Other aversive conditioned stimuli, however, have been shown to increase dialysate DA in the NAc (Young et al., 1993; Wilkinson, 1994) as did the CS⁺ that had been paired previously with footshock in the present study. Further work will be necessary to establish the precise nature of the stimulus events that lead to either increases or decreases in extracellular DA in the NAc or other brain regions. The present studies demonstrate only that combinations of startle response testing and *in vivo* microdialysis sampling provide feasible approaches to addressing such questions.

Similarly, the mechanisms underlying the inhibition of startle-induced decreases in NAc DA by antecedent prepulse stimuli, which mirror the phenomenon of prepulse inhibition of startle itself, remain unclear. It is unlikely that the prepulse stimuli elicit direct changes in the activity of DA neurons that mediate the behavioral or neurochemical effects of the prepulses on startle

Figure 6. Changes in dialysate DA levels in animals receiving the same blocked, pulse-alone, and prepulse trials detailed in Figure 4. Data are shown for individual animals ($n = 10$) and are expressed as the difference, in fmol per 6 min sampling bin, between dialysate DA during and immediately after the blocks of startle stimuli and the mean of the preceding two baseline samples. The bars represent the mean difference in dialysate DA levels for a given sampling bin.

Figure 7. Changes in dialysate DA levels in animals receiving a single presentation of a 30 sec visual (2.5 Hz flashing light) aversive CS⁺ within the startle chamber. The flashing light had been paired with footshock (0.5 mA/0.5 sec) in a separate environment the previous day. Again, data are shown for individual animals ($n = 6$) and are expressed as the difference, in fmol per 6 min sampling bin, between dialysate DA during and immediately after the CS⁺ and the mean of the preceding two baseline samples. The bars represent the mean difference in dialysate DA levels for a given sampling bin.

responses (cf. Swerdlow et al., 1992). Instead, dopaminergic influences appear to modulate brainstem mechanisms that subserve prepulse inhibition. Hence, it is likely that the prepulse-induced inhibition of the effects of startling stimuli on NAc DA levels similarly reflects an influence of more rapidly responding neuronal systems on or via interactions with dopaminergic systems. For example, glutamatergic afferents to the NAc are believed to modulate presynaptic dopaminergic activity (Youngren et al., 1993) and to influence prepulse inhibition (Caine et al., 1991; Wan et al., 1995). Moreover, as illustrated by the disruption of prepulse inhibition produced by NMDA antagonists (compare Fig. 2) (Mansbach and Geyer, 1989), manipulations of glutamatergic systems have profound effects on prepulse inhibition. It may be therefore, that simultaneous measurements of DA and glutamate release would be of interest in future studies.

Implications for general constructs of DA function

Previous attempts to correlate regional DA release to particular behaviors suggest that dopaminergic transmission is altered under a variety of circumstances, including appetitive situations, aversive

situations, motor activation, and sensorimotor integration (Salamone, 1994). With regard to the distinction between appetitive and aversive situations, it may be assumed that startling stimuli would fall into the latter category, insofar as startle testing per se can produce increases in circulating levels of corticosterone and ACTH (Glowa et al., 1992). As mentioned above, however, other aversive stimuli, either unconditioned or conditioned, typically lead to increases rather than decreases in measures of extracellular DA in the NAc. Thus, it seems likely that some aspect of startling stimuli other than their presumed aversiveness is responsible for their ability to decrease NAc DA.

The relationships between motoric activation and NAc DA were more internally consistent within the present study. That is, robust motoric activity in the form of whole-body startle responses was associated with decreases in NAc DA, whereas inhibited startle responses were associated with relatively higher DA levels. Such an inverse relationship between motor output and NAc DA, however, is inconsistent with several other reports of either no change or an increase in measures of extracellular DA in the NAc when motoric activity is activated (Damsma et al., 1992; Hattori et al., 1994). Furthermore, in the present study, the motor responses to startling stimuli exhibited habituation, whereas the neurochemical effects did not. Hence, it would be difficult to argue for any general relationship between levels of motoric activity and the release of DA in the NAc.

The basic idea that NAc DA release is related to sensorimotor integration provided much of the original rationale for the present investigation. In particular, the involvement of mesolimbic dopaminergic systems in the modulation of prepulse inhibition, often considered to be an example of sensorimotor gating mechanisms, is extensive (see above). The concept of sensorimotor integration, however, subsumes a wide range of processes (e.g., habituation, gating, stimulus detection, stimulus salience) that have yet to be examined adequately in relationship to the dynamics of NAc dopaminergic terminals. The present findings provide additional support for the involvement of NAc DA in one form of sensorimotor gating, namely prepulse inhibition, but leave unanswered questions regarding other processes involved in sensorimotor integration. Nevertheless, this investigation suggests that it will be practical to apply *in vivo* microdialysis sampling methods to the several powerful paradigms in which measures of startle responses are used to assess fundamental aspects of sensorimotor processing and behavioral plasticity.

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Figure 8. Changes in dialysate DA levels in animals receiving 1.5 mg/kg D-amphetamine. The drug was administered intraperitoneally in the holding cage at the end of the startle chamber session. Data are the mean difference $\pm$ SEM ($n = 9$), in fmol per 6 min sampling bin, between dialysate levels subsequent to systemic D-amphetamine and the mean of the two preceding baseline samples.

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