

# Glutamate Receptor-Dependent Modulation of Dopamine Efflux in the Nucleus Accumbens by Basolateral, But Not Central, Nucleus of the Amygdala in Rats

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Dopaminergic neurotransmission in the nucleus accumbens (NAc) and neural processes in the basolateral (BLA) and central (CeN) amygdala nuclei are implicated in associative reward learning. Given their direct and indirect connections with the NAc and ventral tegmental area (VTA), both the BLA and CeN may regulate the mesoaccumbens dopamine (DA) system in rewarding situations. Electrical stimulation of the BLA (20 Hz, 10 sec, 300  $\mu$ A) induced a long-lasting  $25 \pm 4\%$  increase in DA efflux in the NAc, measured by microdialysis in freely moving rats, whereas comparable stimulation of the CeN had no effect. Reverse dialysis of either the NMDA receptor antagonist APV (100  $\mu$ M) or the AMPA–kainate receptor antagonist DNQX (100  $\mu$ M), but not the metabotropic glutamate receptor antagonist ( $\pm$ )-amino-4-carboxy-methyl-phenylacetic acid (100  $\mu$ M), into

the NAc blocked the stimulation-evoked increase in DA efflux in the NAc. VTA infusion of lidocaine (lido; 4%) significantly reduced basal DA levels for  $\sim 30$  min but failed to suppress the increase in NAc DA efflux resulting from BLA stimulation. Additionally, infusions of lido (4%) into the medial prefrontal cortex failed to block the stimulation-evoked increase in NAc DA efflux. These data support the hypothesis that the BLA can directly modulate DA efflux through local mechanisms in the NAc, independent of an action on DA cell bodies in the VTA. The finding that brief activation of the CeN had no long-lasting effects on DA efflux in the NAc suggests an important degree of functional independence between the CeN and BLA.

**Key words:** basolateral amygdala; central amygdala; dopamine; nucleus accumbens; glutamate; reward

The nucleus accumbens (NAc) plays a critical role in the acquisition and expression of appropriate behavioral responses to rewarding stimuli (Everitt et al., 1999, 2000; Ikemoto and Panksepp, 1999; Parkinson et al., 2000a). Reward-related information may be relayed to the NAc via glutamatergic inputs from the amygdala, hippocampus, and medial prefrontal cortex (Groenewegen et al., 1991; Brog et al., 1993; Meredith and Totterdell, 1999). Additionally, the NAc receives a dense dopaminergic projection originating in the ventral tegmental area (VTA) of the midbrain (Björklund and Lindvall, 1984). A growing body of evidence indicates that mesoaccumbens dopamine (DA) is an important neural modulator of both NAc medium spiny neurons (Harvey and Lacey, 1997; Hernández-López et al., 1997; Nicola et al., 2000) and their corticolimbic inputs (Wickens et al., 1996; Floresco et al., 2001a,b). Thus, DA neurotransmission may bias responses of the NAc to particular inputs that in turn may facilitate appropriate behavioral response selection in complex, rewarding situations (Mogenson et al., 1993; Ikemoto and Panksepp, 1999; Redgrave et al., 1999; Floresco et al., 2001a,b).

On the basis of behavioral and neurochemical experiments, the amygdala appears to regulate NAc DA efflux in rewarding envi-

ronments (Cador et al., 1989; Robledo et al., 1996; Floresco et al., 1998, 2001b; Everitt et al., 1999, 2000). The basolateral amygdala (BLA) sends a dense glutamatergic projection to the NAc (Kelley et al., 1982; Brog et al., 1993; Wright et al., 1996) that synapses in close apposition to mesoaccumbens DA varicosities (Johnson et al., 1994). The BLA and NAc interact during the formation and expression of stimulus–reward associations (Everitt et al., 1991; Robbins and Everitt, 1992; Baldwin et al., 2000), and afferent activity from the BLA can increase DA efflux via glutamatergic NMDA receptors in the NAc, which facilitates subsequent input from the BLA (Floresco et al., 2001b). The BLA may also regulate NAc DA efflux indirectly by altering DA cell body firing in the VTA via glutamatergic afferents to the medial prefrontal cortex (mPFC), which in turn projects to the VTA (Kelley et al., 1982; Jackson and Moghaddam, 2001).

The central nucleus of the amygdala (CeN) is often considered to be the main output nucleus of the amygdala, primarily on the basis of its GABAergic efferents to midbrain autonomic sites (Krettek and Price, 1978; Swanson and Petrovich, 1998). The CeN plays a critical role in both aversive and appetitive Pavlovian conditioning paradigms (Davis, 1992; Hatfield et al., 1996; Killcross et al., 1997; Parkinson et al., 2000b). Additionally, CeN lesions disrupt the potentiation of conditioned reinforcement by intra-accumbens amphetamine infusions (Robledo et al., 1996), which is dependent on mesoaccumbens DA transmission (Taylor and Robbins, 1986). The CeN may interact with the mesoaccumbens DA system through a direct GABAergic projection to the VTA (Wallace et al., 1992; Everitt et al., 1999, 2000), although other indirect routes are possible (Zahm, 2000). Taken together, these data suggest that the BLA and CeN may regulate mesoac-

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cumbens DA efflux differentially, thereby influencing the selection of appropriate response patterns to rewarding stimuli.

The present study used *in vivo* microdialysis in freely moving rats to examine the modulation of DA efflux in the NAc by brief stimulation of either the BLA or CeN. Given the findings of Floresco et al. (1998) in anesthetized rats and the data of Everitt and colleagues (Robledo et al., 1996; Everitt et al., 1999, 2000), we hypothesized that high-frequency electrical stimulation of either the BLA or CeN would increase DA efflux in the NAc. Stimulation parameters were based on single-unit recording studies demonstrating that amygdalar neurons fire at frequencies ranging from 10 to 40 Hz when rats are presented with either natural or conditional rewarding stimuli (Muramoto et al., 1993; Uwano et al., 1995; Pratt and Mizumori, 1998). Reverse dialysis of selective glutamate receptor antagonists into the NAc and microinfusions of lidocaine (lido) into either the VTA or mPFC were combined with BLA stimulation in separate experiments to examine the circuitry underlying the effects of stimulation on DA efflux in the NAc.

## MATERIALS AND METHODS

**Subjects.** Male Long-Evans rats (Charles River Canada, St. Constant, Quebec, Canada) were used in all experiments. Rats were housed individually with *ad libitum* access to food (Purina Rat Chow) and water. All rats were maintained on a 12 hr light/dark cycle (lights on at 7:00 A.M.). Experiments were conducted in strict accordance with the standards of the Canadian Council on Animal Care and were approved by the Committee on Animal Care at the University of British Columbia.

**Surgery.** Surgery was performed on rats weighing 320–400 gm. All rats were anesthetized with ketamine hydrochloride (100 mg/kg, i.p.; MTC Pharmaceuticals) and xylazine (10 mg/kg, i.p.; Rompun). They were then placed in a stereotaxic apparatus (Kopf, Tujunga, CA), the dorsal skull surface was exposed, and holes were drilled. A bipolar stimulating electrode with the tips spread to a diameter of 0.5 mm (Plastics One, Roanoke, VA) was implanted into either the BLA [anteroposterior (AP) –3.4 mm from bregma, mediolateral (ML)  $\pm$  5.0 mm from the midline, dorsoventral (DV) –7.6 mm from dura] or CeN (AP –2.4 mm, ML  $\pm$  4.2 mm, DV –7.0 mm), and a guide cannula (19 gauge, 15 mm) was implanted dorsal to the ipsilateral NAc shell (AP +1.8 mm, ML  $\pm$  1.1 mm, DV –1.0 mm). Rats used in the VTA lidocaine (lido) experiment also had a guide cannula (23 gauge, 20 mm) implanted dorsal to the ipsilateral VTA (AP –4.8 mm, ML  $\pm$  1.0 mm, DV –6.5 mm), whereas rats used in the mPFC lido experiment had guide cannulas (23 gauge, 13 mm) implanted dorsal to the mPFC bilaterally (AP +3.4 mm, ML  $\pm$  0.7 mm, DV –2.5 mm). All coordinates were calculated using the rat brain atlas of Paxinos and Watson (1997). Cannulas and the electrode were secured to the skull with four jeweler's screws and dental acrylic. Wire obdurators were inserted into the cannulas to keep them patent. Rats were given at least 5 d to recover from surgery before testing.

**Microdialysis procedure.** Concentric-style microdialysis probes (2 mm of exposed membrane) were constructed in our laboratory (Taepavarapruk et al., 2000). Probes were attached to gas-tight syringes (Hamilton, Reno, NV) via a liquid swivel (Instech Inc., Plymouth Meeting, PA) containing perfusion medium (147 mM NaCl, 3.0 mM KCl, 1.3 mM CaCl<sub>2</sub>·H<sub>2</sub>O, 1.0 mM MgCl<sub>2</sub>·0.6H<sub>2</sub>O, and 0.01 sodium phosphate buffer, pH 7.3–7.4) and flushed for 10–20 min using a syringe pump (model 22; Harvard Apparatus, South Natick, MA). Probes were then secured in a copper collar and inserted into the NAc (7.8 mm ventral to dura), and the rats were allowed to move freely in a Plexiglas box (32 cm  $\times$  32 cm  $\times$  41 cm high) with access to food and water for 12–18 hr before experimental testing began the following morning. The probes were continuously perfused at 1  $\mu$ l/min overnight.

**Analysis of dialysate samples.** Two HPLC systems with electrochemical detection (HPLC-ED) consisting of an ESA 582 pump (ESA Inc., Bedford, MA), Rheodyne (Rohmert Park, CA) Inert manual injector, an Ultrasphere column (Beckmann, Fullerton, CA) (ODS 5  $\mu$ m, 15 cm  $\times$  4.6 mm), an ESA 5011 analytical cell, and a Coulochem II EC detector (ESA Inc.) were used to quantify DA levels in all experiments. The working potentials were: +450 mV (electrode 1), –300 mV (electrode 2), and +450 mV (guard cell). The mobile phase (pH 3.5) consisted of 6 gm/l sodium acetate, 10 mg/l EDTA, 150 mg/l octyl sulfate (adjustable),

35 ml/l glacial acetic acid, and 865 ml of Milli Q purified water. After it was filtered through a 0.22  $\mu$ m sterile nylon filter unit (Millipore, Bedford, MA), methanol (HPLC grade, 10% of total volume, adjustable) was added, and the mobile phase was degassed before use. Chromatograms were registered on a dual-pen chart recorder (Kipp and Zonen, Bohemia, NY). All samples were injected immediately after collection, and DA peak heights were measured manually.

**Experimental procedure.** A within-subjects design was used in all experiments, and this was combined with a between-subjects design for the VTA and mPFC lido experiments (see below). Samples were collected every 10 min throughout the experiment and after four baseline samples were collected that did not differ by more than  $\pm$ 10%, the rats received electrical stimulation. Cathodal constant current pulses were delivered to either the BLA or CeN through an isolator (Iso-flex; A.M.P.I., Jerusalem, Israel) via a Master-8 stimulator (A.M.P.I.). Two hundred pulses (0.4 msec duration) were delivered at an intensity of 300  $\mu$ A and a frequency of 20 Hz over 10 sec. The intensity of stimulation was based on a similar protocol used for stimulation of the ventral hippocampus in our laboratory (Taepavarapruk et al., 2000). Stimulation was timed to ensure that the next dialysis sample reflected only “stimulation-evoked” changes in DA efflux. The experimenter remained in the testing room after the stimulation to record any behavioral effects of the stimulation.

**Pharmacological experiments in conjunction with BLA stimulation.** In separate groups of rats, reverse dialysis was used to deliver the AMPA-kainate receptor antagonist 6,7-dinitroquinoxaline-2,3-dione (DNQX), the NMDA receptor antagonist ( $\pm$ )-2-amino-5-phosphonopentanoic acid (APV), or the broad spectrum metabotropic glutamate receptor (mGluR) antagonist (+)- $\alpha$ -methyl-4-carboxyphenylglycine (MCPG) to the NAc through the same probe used to collect samples. The dose of each drug in the perfusion medium was 100  $\mu$ M. Previous experiments (Taepavarapruk et al., 2000) (P. Taepavarapruk and A. G. Phillips, unpublished observations) indicated that an intraprobe concentration of 100  $\mu$ M of each receptor antagonist is sufficient to block glutamatergic receptor activation effectively in the NAc. Perfusion of the drug into the NAc started 20 min before the second stimulation of the BLA and continued for a total of 60 min.

**Lidocaine microinfusion experiments in conjunction with BLA stimulation.** Infusion needles were constructed from 30 gauge stainless steel tubing and PE-50 (Intramedic; Becton Dickinson, Mountain View, CA) tubing. The needles were filled with a 4% lido solution before being inserted into the VTA or mPFC. All needles were inserted 1 mm past the end of the cannulas into the VTA or mPFC at least 2 hr before the experiment began. The microinfusion needles remained in place throughout the experiment.

Before the infusions, the needles were connected to gas-tight syringes (500  $\mu$ l; Hamilton, Reno, NV). The desired volume of lido (VTA: 2  $\mu$ l, ipsilateral to the electrode; mPFC: 1  $\mu$ l, bilateral) was infused (0.5  $\mu$ l/min) via a syringe pump (model 22; Harvard Apparatus) 10 min before the second stimulation. The experimenter confirmed the progress of the infusion by measuring the movement of an air bubble in the PE-50 line during the infusion. Five minutes after the infusion ended, rats in the stimulation groups were stimulated a second time, whereas rats in the control groups were not (see Results). The effect of lido as a reversible Na<sup>+</sup> channel blocker is greatest ~5 min after infusion and may last for up to 30 min (Tehovnik and Sommer, 1997). Dialysate samples were collected for 60 min after the second stimulation in all experiments.

**Drug preparation.** Appropriate amounts of APV, DNQX, and MCPG (Precision Biochemical, Vancouver, Canada) were dissolved in a drop of NaOH and sterile H<sub>2</sub>O to create a 10 mM stock solution (volume was adjusted to 1 ml with perfusion medium; pH was adjusted to ~7.0). The stock solutions were then diluted to 100  $\mu$ M with perfusion medium and stored at –20°C until use. Lido (4%) was made freshly before each experiment by dissolving 20 mg/ml of lido HCl powder (Research Biochemicals, Natick, MA) into an injectible 2% lido solution (Ayerst, Guelph, Ontario, Canada).

**Histology.** After the experiments, all rats were injected with a lethal dose of chloral hydrate and perfused transcardially with 0.9% saline followed by 10% formaldehyde. Brains were stored in 10% sucrose in 10% formaldehyde for at least 1 week, after which they were sectioned (50  $\mu$ m) using a cryostat and stained for Nissl substance with cresyl violet. Placements of the probes, electrodes, and infusion needles were verified under a light microscope with the assistance of a rat brain atlas (Paxinos and Watson, 1997).

**Data analysis.** The height of each DA peak was measured directly from the chromatograms, and baseline DA levels for each rat were calculated

by averaging the first three samples included in the analysis. In all figures, DA levels are expressed as a percentage of the baseline. One-way repeated measures ANOVAs with time as a within-subjects factor were performed with the aid of SPSS (version 10.0) on all data, and Dunnett's *post hoc* tests were performed where appropriate. The baseline sample taken immediately before the first stimulation was used as the critical value during computation of the Dunnett's *post hoc* tests. To assess differences between groups in the lido experiments, planned contrasts between samples for time points of interest were computed, and significance was determined using the Bonferroni correction for  $\alpha$  levels.

## RESULTS

### High-frequency stimulation of the BLA, but not the CeN, induces a long-lasting increase in NAc DA efflux

As shown in Figure 1A, baseline DA levels were very stable before brain stimulation. High-frequency stimulation (20 Hz; 10 sec; 300  $\mu$ A;  $n = 7$ ) of the BLA induced a significant ( $F_{(21, 126)} = 3.68$ ;  $p < 0.0001$ ; Dunnett's,  $p < 0.05$ ) increase in DA efflux in the NAc that returned to baseline 30 min after the stimulation (Fig. 1A). A second train of brain stimulation 2 hr (12 samples) after the first stimulation also produced a significant increase in DA efflux (Dunnett's,  $p < 0.05$ ). DA levels returned to baseline 40 min after stimulation, and the experiment was terminated 60 min after the second stimulation.

Throughout testing, rats generally slept and rested except for exhibiting a number of characteristic behavioral responses immediately after stimulation of the BLA. Behavioral responses to stimulation are summarized in Table 1. The only unexpected behavioral response to the stimulation was a characteristic rapid turn between 90° and 270° during the stimulation exhibited by 35% of the rats receiving BLA stimulation. In 87% of these rats, the turn was ipsilateral to the electrode. The behavioral responses exhibited by a given rat were similar after the first and second stimulation and during the pharmacological and lido experiments. Thus, it is likely these behavioral changes were caused by activation of the amygdala and were unrelated to the changes in DA efflux observed in the NAc.

Histological analyses from all experiments involving BLA stimulation confirmed that a significant stimulation-evoked increase in DA efflux in the NAc was observed only when electrode placements were located in the caudal BLA ( $n = 46$ ) (Fig. 1C). The majority of effective placements (shown on the left side of the sections) were centered in the caudal magnocellular accessory basal and parvocellular basal nuclei (−3.3 mm, −3.6 mm, and −3.8 mm from bregma). The right side of each section depicts a representative sample of placements from rats which did not show an increase in DA efflux in the NAc when stimulated ( $n = 30$ ). These placements were located in the lateral nucleus of the amygdala ( $n = 11$ ), ventral piriform cortex ( $n = 8$ ), CeN ( $n = 4$ ), bed nucleus of the stria terminalis ( $n = 5$ ), and caudate putamen ( $n = 2$ ). Six rats represented by placements shown on the right side of this figure served as the stimulated control group (Stimctrl + lido) in the VTA–lido experiment discussed below. Representative placements of the microdialysis probes used in all experiments are shown in Figure 1D. Many of the probes were centered on the border between the shell and core of the NAc, and sampled primarily from areas where BLA afferents terminate.

In contrast to the data obtained with placements in the BLA, electrical stimulation of the CeN (20 Hz; 10 sec; 300  $\mu$ A;  $n = 6$ ) failed to produce a significant change in DA efflux in the NAc (data not shown;  $F_{(9, 45)} = 1.32$ ; NS). To ensure that this finding was not caused by insufficient current intensity, an additional group of eight animals were implanted with electrodes in the CeN

and stimulated at intensities of 300, 450, 600, and 800  $\mu$ A with 60 min between each stimulation. Dialysis samples were collected continuously for the duration of this experiment, and no significant change in NAc DA efflux was detected at any of these current intensities (Fig. 1B) ( $F_{(27, 189)} = 1.34$ ; NS). It is interesting to note that after the 800  $\mu$ A stimulation (Fig. 1B, S4), there was a small nonsignificant increase in DA efflux in the NAc. This effect may be attributable to the spread of the high current intensity that activated a portion of the BLA in some animals. Behavioral effects of CeN stimulation were similar to those observed after BLA stimulation and are also described in Table 1. Rats in the parametric study (300–800  $\mu$ A) displayed an increase in the duration of chewing behaviors at higher current intensities. Stimulating electrodes in the CeN group were located in the lateral and medial divisions of the CeN ( $n = 14$ ) (Fig. 1D).

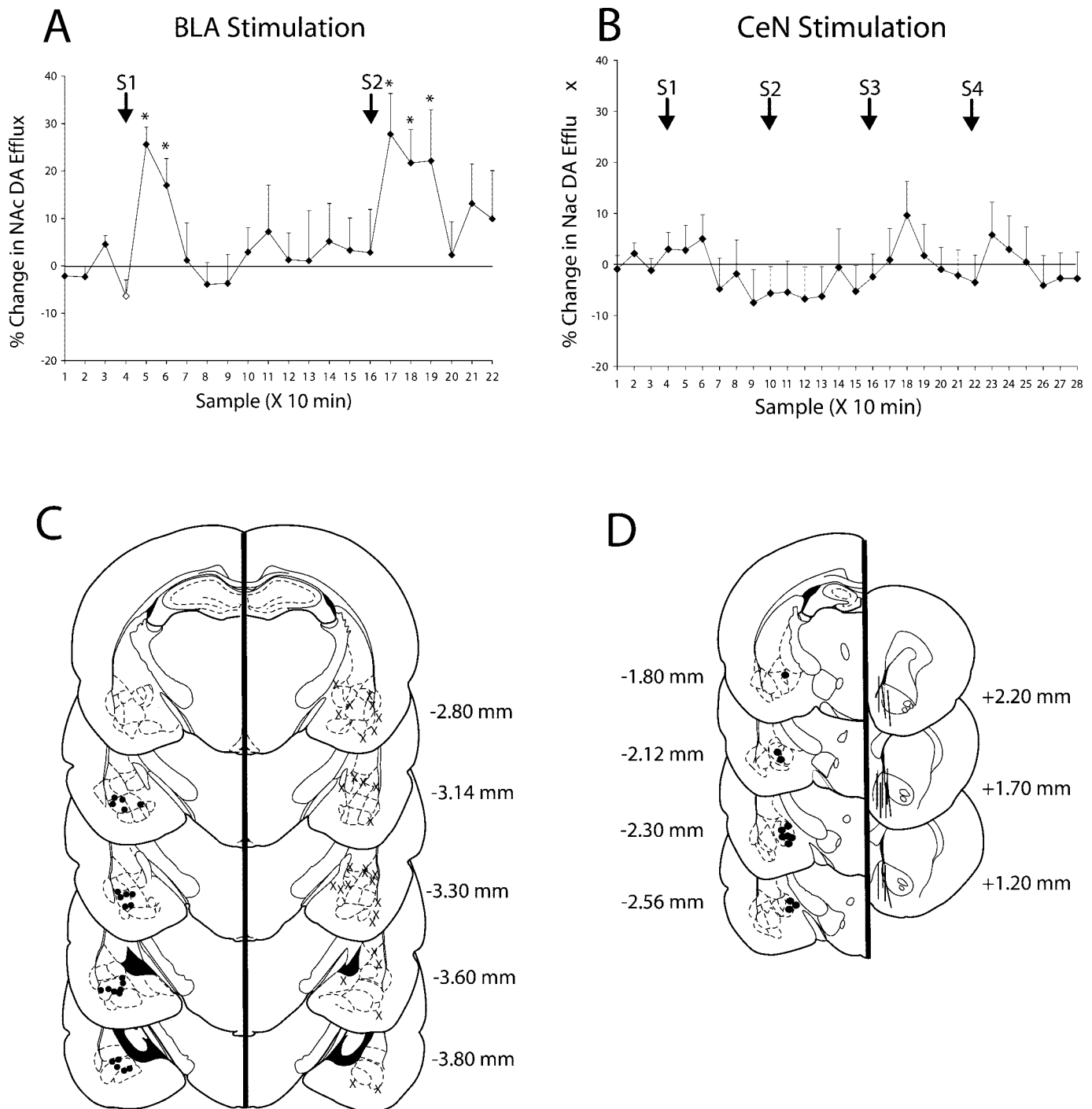
### Increased NAc DA efflux after stimulation of the BLA is dependent on ionotropic glutamate receptors located in the NAc

Reverse dialysis experiments examined the role of both ionotropic and metabotropic glutamate receptors in the NAc in the effects of BLA stimulation on NAc DA efflux. Control stimulation of the BLA before delivery of the ionotropic glutamate receptor (iGluR) antagonists APV ( $n = 7$ ) or DNQX ( $n = 8$ ) resulted in a significant increase in DA efflux in the NAc similar to that observed above (Fig. 2) (APV group:  $F_{(21, 126)} = 2.39$ ,  $p < 0.005$ ; Dunnett's,  $p < 0.05$ ; DNQX group:  $F_{(21, 147)} = 4.44$ ,  $p < 0.0001$ ; Dunnett's,  $p < 0.05$ ). Reverse dialysis of either APV (100  $\mu$ M) or DNQX (100  $\mu$ M) for 20 min before, and 40 min after, the second BLA stimulation blocked the increase in DA efflux (Fig. 2, Dunnett's, NS). In contrast, reverse dialysis of the mGluR antagonist MCPG (100  $\mu$ M) failed to block BLA stimulation-evoked increase in DA efflux ( $n = 6$ ;  $F_{(21, 105)} = 2.05$ ;  $p < 0.01$ ; Dunnett's,  $p < 0.05$  for both the first and second stimulation).

Application of these drugs did not change the behavior of the rats or significantly affect basal DA levels. However, as can be seen in Figure 2, reverse dialysis of DNQX into the NAc did cause a small, nonsignificant ( $10 \pm 6\%$ ) decrease in basal DA levels in the NAc 10 min after administration began (immediately preceding stimulation 2). This effect has been seen previously (Taepavarapruk et al., 2000) and may reflect a role for AMPA–kainate receptors located in the NAc in regulating basal levels of DA release.

### Infusion of lidocaine into the VTA significantly reduced basal DA levels in the NAc but failed to attenuate the BLA stimulation-evoked increase in NAc DA efflux

The next experiment assessed the contribution of DA cell body firing in the VTA to the increase in NAc DA efflux after stimulation of the BLA. The general design was the same as the previous experiments except three groups of rats were used, including one group receiving lido alone. After baseline sampling, the group receiving BLA stimulation before lido treatment (BLAstim + lido group;  $n = 9$ ) showed the characteristic, significant increase in NAc DA efflux (Fig. 3A) ( $F_{(18, 342)} = 13.4$ ;  $p < 0.0001$ ; Dunnett's test,  $p < 0.05$ , BLAstim + lido group only; between groups contrast:  $t_{(19)} = 2.73$ ;  $p < 0.01$ ) as observed in the previous experiments. Brain stimulation at control sites (Stimctrl + lido;  $n = 6$ ) failed to evoke an increase in NAc DA efflux (Dunnett's test, NS). As shown in Figure 1C, stimulation of only the caudal BLA resulted in a significant increase in DA efflux in the NAc. Histological analysis revealed that the stimulating electrodes in the Stimctrl + lido group were positioned outside this



**Figure 1.** Effects of high-frequency stimulation of the BLA and CeN on DA efflux in the NAc. **A**, High-frequency stimulation of the BLA (20 Hz, 10 sec,  $n = 7$ , S1) followed 2 hr later by a second train of stimulation (20 Hz, 10 sec, S2). Asterisks denote significant differences from baseline (white diamond) at the level of  $p < 0.05$ . Data points represent a mean level of DA obtained over a 10 min sampling period (see Materials and Methods for details), and error bars represent SEM (for both **A** and **B**). **B**, High-frequency stimulation (20 Hz, 10 sec) of the CeN at four different current intensities (S1 = 300  $\mu$ A, S2 = 450  $\mu$ A, S3 = 600  $\mu$ A, S4 = 800  $\mu$ A) failed to induce significant changes in DA efflux ( $n = 8$ ). **C**, Representative placements of electrodes of effective (left side, black circles) and ineffective (right side, black "x") sites for inducing an increase in DA efflux, in all experiments. For clarity not all placements are shown. Numbers correspond to the anterior or posterior distance (in millimeters) of each plate from bregma (both **C** and **D**). **D**, Representative placements of electrodes aimed at the CeN (left side, black circles) and microdialysis probes aimed at the NAc (right side). The black bars illustrate the position of the probes and are scaled to 2 mm in length to accurately reflect only the area of the brain from which each probe sampled. Plates adapted from Paxinos and Watson (1997).

area; therefore, this group provided a control for the generalized effects of stimulating areas surrounding the BLA on NAc DA efflux in conjunction with the VTA lido infusion.

Microinfusion of lido into the VTA (2  $\mu$ l, 0.5  $\mu$ l/min, ipsilateral to the electrode) significantly reduced basal DA levels in all three groups ( $F_{(18, 342)} = 13.4$ ;  $p < 0.0001$ ; Dunnett's,  $p < 0.05$ , all

**Table 1. Behavioral responses of rats to stimulation of the BLA or CeN**

Behaviors	BLA ( <i>n</i> = 46) (%)	CeN ( <i>n</i> = 14) (%)
Awaken	100	100
Mouth movements/chewing	82	64
Rapid turn during stimulation	35	21
Return to sleeping/resting		
(<2 min)	25	36
(>2 min, <5 min)	64	43
(>5 min)	11	21
Wet dog shake	16	0

Number of rats displaying a particular behavior is expressed as a percentage of the total number of rats receiving stimulation.

groups) consistent with a blockade of Na<sup>+</sup> channels by lido that in turn reduced firing of DA neurons in the VTA. As can be seen from Figure 3A, DA efflux in the three groups was reduced by 35–40%, 10 min after infusion of lido. DA levels in the lido group remained significantly below baseline for the next 20 min (Dunnett's,  $p < 0.05$ ) reaching a nadir of  $54 \pm 4\%$  below baseline 20 min after the infusion of lido was terminated. As expected, stimulation at control sites in the Stimctrl + lido group after the lido infusion did not change DA levels relative to the lido group, and levels of DA efflux remained significantly below baseline values for a total of 30 min (Dunnett's,  $p < 0.05$ ). The 30 min duration of this effect reflects the typical time course of lido in brain tissue (Tehovnik and Sommer, 1997) and confirms the success of the infusions.

In contrast to the data from the two control groups, brain stimulation in the BLAstim + lido group significantly increased NAc DA efflux relative to the attenuated baseline 10 min after the lido infusion (Dunnett's,  $p < 0.05$ ). DA efflux in the BLAstim + lido group was 42% higher than the two control groups (Fig. 3B), however, values were not increased above baseline. DA efflux in the BLAstim + lido group remained significantly higher than control values for at least 10 min ( $t_{(19)} = 8.94$ ;  $p < 0.0005$ ), after which the control values returned to baseline levels. Placements of the microinfusion cannulas aimed at the VTA in this experiment are shown in Figure 3C.

#### Bilateral infusions of lidocaine into the mPFC fail to affect the BLA stimulation-evoked increase in NAc DA

Using a design similar to that of the VTA lido experiment described above, the final experiment assessed the possible contribution of the glutamatergic projection from the BLA to the mPFC in modulating the effects of BLA stimulation on NAc DA efflux. As is shown in Figure 4, initial stimulation of the BLA (BLAstim + lido group;  $n = 9$ ) induced a significant  $23 \pm 5\%$  increase in NAc DA efflux ( $F_{(18, 288)} = 2.06$ ,  $p < 0.01$ , Dunnett's,  $p < 0.05$ ; between groups contrast:  $t_{(16)} = 4.17$ ,  $p < 0.0005$ ) that remained significantly above baseline levels for 60 min. Bilateral infusion of lido (1  $\mu$ l/side) into the mPFC was accompanied by a small reduction in basal DA release in the control group (lido group;  $n = 9$ ) for 10 min after infusion (Fig. 4).

Infusion of lido bilaterally into the mPFC failed to block the BLA stimulation-evoked increase in NAc DA efflux (Dunnett's,  $p < 0.05$ ; between groups contrast:  $t_{(16)} = 2.70$ ,  $p < 0.01$ ), suggesting that the BLA afferents to the mPFC do not contribute significantly to the increases in NAc DA efflux after BLA stimulation. The infusion cannulas of the rats used in this experiment

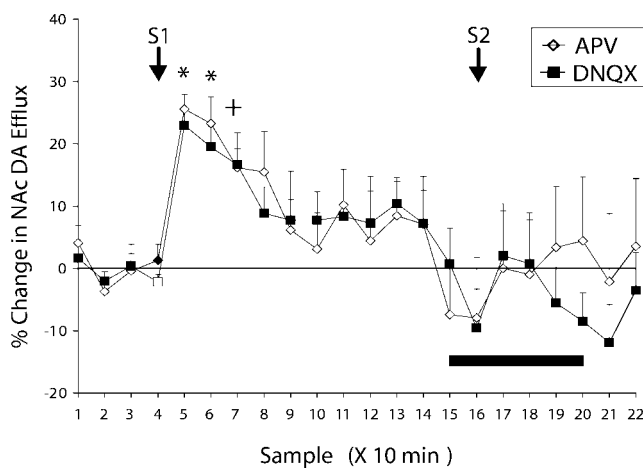
were positioned within the infralimbic and prelimbic areas of the mPFC (Fig. 3C).

## DISCUSSION

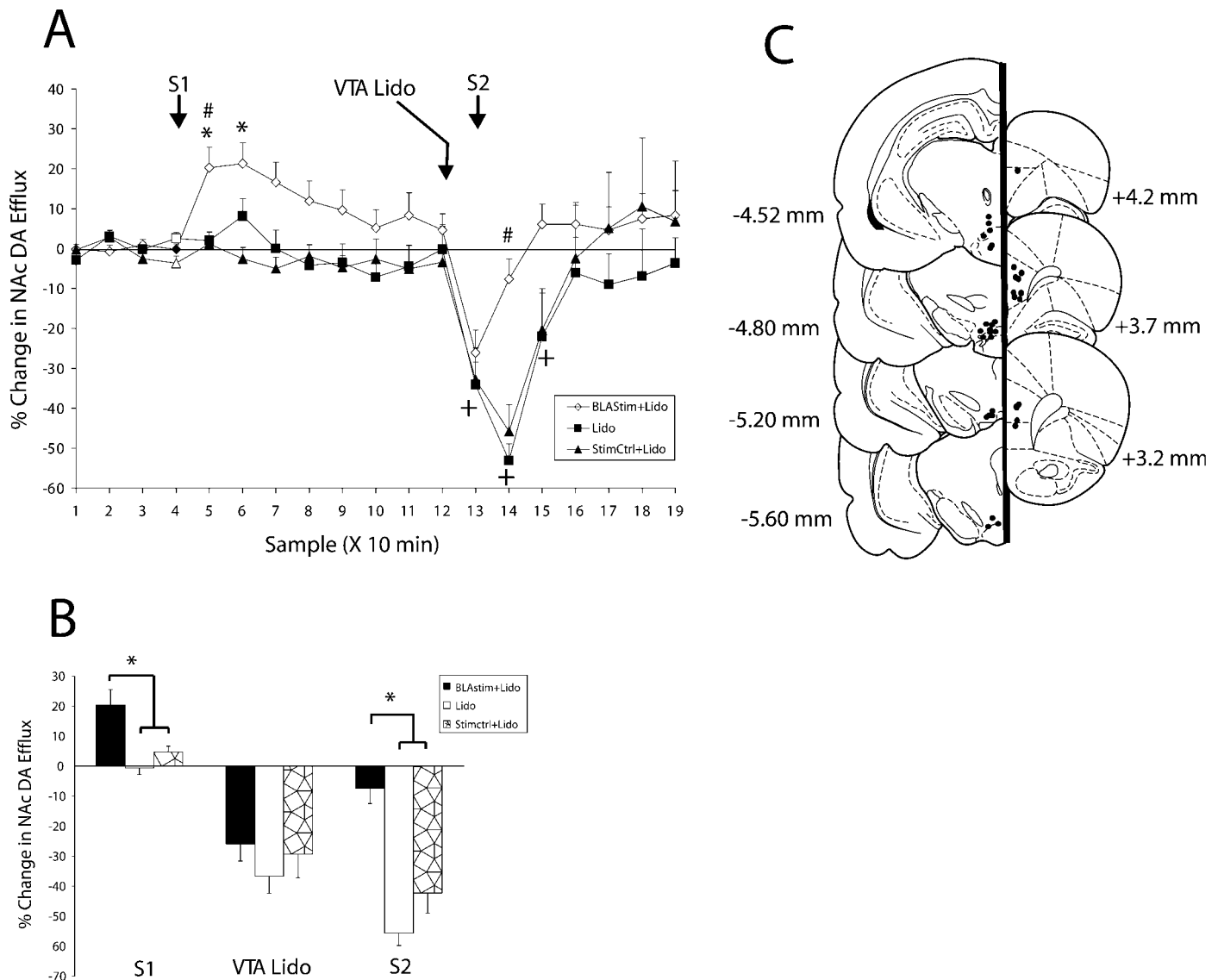
Brief, high-frequency stimulation of the BLA caused a long-lasting increase in DA efflux in the NAc of awake, unrestrained rats. Both the magnitude and duration of this increase is similar to that observed in the NAc of rats presented with natural rewards such as food (Ahn and Phillips, 1999). In contrast, stimulation of the CeN at similar and higher current intensities did not produce significant changes in DA efflux. After stimulation of either nucleus, the animals displayed increased levels of activity and alertness for a number of minutes. Reverse dialysis of the iGluR antagonists APV and DNQX, but not the mGluR antagonist MCPG, into the NAc blocked the BLA stimulation-evoked increase in NAc DA efflux. Histology results, summarized in Figure 1C, indicate that stimulation of only the caudal BLA resulted in increased NAc DA efflux, consistent with the location of a direct glutamatergic projection to the NAc (Kelley et al., 1982; Brog et al., 1993; Wright et al., 1996). Inactivation, with the Na<sup>+</sup> channel blocker lido, of either the mesoaccumbens DA cell bodies in the VTA, or the region of the mPFC that receives a glutamatergic projection from the BLA, failed to block the BLA stimulation-evoked increase in DA efflux in the NAc. These results complement data from the anesthetized preparation (Floresco et al., 1998) and suggest that the BLA may modulate NAc DA efflux via a local mechanism in the NAc.

#### Distinct patterns of NAc DA modulation by the BLA and CeN

The BLA and CeN interact with the mesoaccumbens DA system via different pathways, and this may partially account for the different effects on NAc DA efflux observed after stimulation of these amygdalar nuclei. For example, the BLA sends a direct



**Figure 2.** Reverse dialysis of ionotropic glutamate receptor antagonists into the NAc and the effect of BLA stimulation on NAc DA efflux. Stimulation of the BLA (20 Hz, 10 sec, S1) induced a significant increase in DA efflux in the NAc in both groups ( $p < 0.05$ ). Reverse dialysis of either the NMDA receptor antagonist APV (100  $\mu$ M,  $n = 7$ ; white diamonds) or the AMPA–kainate receptor antagonist DNQX (100  $\mu$ M,  $n = 8$ ; black squares) blocked the expected increase in DA efflux after the second (S2) stimulation of the BLA (20 Hz, 10 sec). Asterisks denote a significant difference from baseline (APV, black diamond; DNQX, white square) for both groups ( $p < 0.05$ ), whereas the cross denotes a significant difference from baseline for only the DNQX group. Error bars represent SEM, and the horizontal bar represents the time period during which APV or DNQX was administered into the NAc by reverse dialysis.

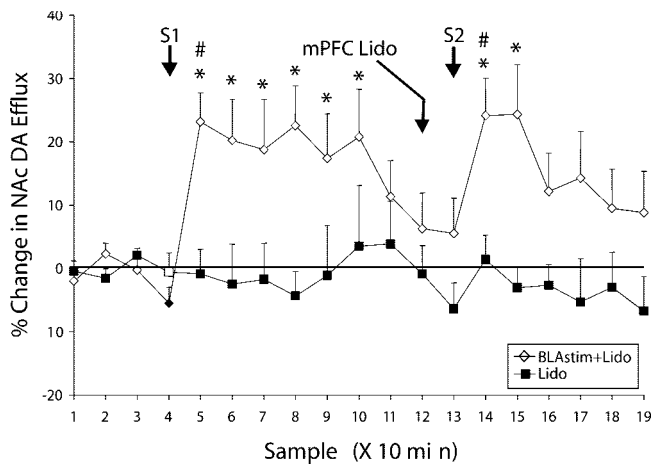


**Figure 3.** Effect of VTA lido infusion on NAc DA efflux evoked by stimulation of the BLA. *A*, Stimulation of the BLA (20 Hz, 10 sec, *S1*) induced a significant increase in DA efflux ( $20 \pm 5\%$ ) in the NAc of the BLAstim + lido group (white diamonds;  $n = 9$ ) but not in the Stimctrl + lido (black triangles;  $n = 6$ ) group. Histological analyses revealed that the electrodes of the Stimctrl + lido group were located outside the caudal BLA (see Results). lido infusion into the VTA of all groups ( $2 \mu\text{l}$ , unilateral, *VTA lido*), caused a significant decrease in basal DA levels in all groups. 10 min after lido infusions, the BLAstim + lido and Stimctrl + lido groups were stimulated a second time (20 Hz, 10 sec, *S2*). After stimulation, DA levels in the NAc of the Stimctrl + lido group were reduced further and were comparable with values of the lido group. DA levels in the BLAstim + lido group were significantly higher than those of the lido and Stimctrl + lido groups for 10 min after the second stimulation (these data are summarized in *B*). Error bars represent SEM. Asterisks denote a significant increase in DA levels above baseline (BLAstim + lido group, black diamond; lido and Stimctrl + lido groups, white square and white triangle, respectively;  $p < 0.05$ ). Crosses indicate a significant decrease in DA levels from baseline ( $p < 0.05$ ). Number signs indicate a significant difference between groups ( $p < 0.001$ ). *B*, Summary of DA levels for the BLAstim + lido (black bars), lido (white bars), and Stimctrl + lido (hatched bars) groups after the first stimulation (*S1*), VTA lido infusion (*VTA-lido*), and the first sample taken after the second stimulation (*S2*). See *A* for details. Asterisks denote a significant difference between groups ( $p < 0.001$ ). *C*, Placements of the microinfusion needles aimed at the VTA (left side) and mPFC (right side) in the lido experiments. See Figure 1C for additional details.

glutamatergic projection to the NAc (Kelley et al., 1982; Brog et al., 1993; Wright et al., 1996) which, given the results of the present experiments, could modulate NAc DA efflux after BLA stimulation. Furthermore, activation of local AMPA–kainate and NMDA receptors in the NAc appears to underlie the changes in NAc DA efflux after BLA stimulation.

Given that brief (10 sec) stimulation of the BLA is sufficient to induce changes in NAc DA efflux lasting for 20–30 min, mechanisms related to short-term potentiation may provide one possible explanation of these effects. Increased levels of intracellular  $\text{Ca}^{2+}$

resulting from NMDA receptor activation and increased  $\text{Ca}^{2+}$ -dependent kinase activity have been implicated in synaptic plasticity (Bliss and Collingridge, 1993; Silva et al., 2000). Increased activity of kinases such as calcium–calmodulin-dependent kinase II and protein kinase A could increase DA efflux in the NAc by phosphorylating ionotropic receptors (Barria et al., 1997), proteins in the soluble *N*-ethylmaleimide-sensitive factor attached protein receptor complex (Risinger and Bennett, 1999), or altering the time course of slow afterhyperpolarizations (Muller et al., 1992; Silva et al., 2000).



**Figure 4.** Effect of bilateral infusion of lido into the mPFC on BLA stimulation-evoked increases in NAc DA. Stimulation of the BLA (20 Hz, 10 sec, S1) induced a significant  $23 \pm 5\%$  increase in NAc DA efflux in the BLAstim + lido group (white diamonds;  $n = 9$ ). Lido infusion into the mPFC in both groups ( $1 \mu\text{l}$ , bilateral, mPFC lido) failed to change basal DA levels significantly in either group (lido group,  $n = 9$ ,  $p > 0.05$  both groups). Stimulation was applied again to the BLAstim + lido group 10 min after the infusion (20 Hz, 10 sec, S2), resulting in another significant  $24 \pm 6\%$  increase in NAc DA efflux. Asterisks denote a significant increase in DA levels above baseline (BLAstim + lido group, black diamond,  $p < 0.05$ ), and number signs indicate significant differences between groups ( $p < 0.001$ ). Error bars represent SEM.

The central and medial nuclei of the amygdala have been described as the origin of the “extended amygdala,” a continuum of neurons that extend into the bed nucleus of the stria terminalis, and through the basal forebrain to the NAc shell (Alheid and Heimer, 1988). Support for a functional role of the extended amygdala in learning and drug abuse has been described (Koob and Le Moal, 1997; Everitt et al., 1999); however, the anatomical complexity of this area makes experimental manipulation difficult. Although there are many anatomical connections within the continuum of neurons connecting the CeN to the NAc shell, the present data indicate that they may not be important in providing long-lasting regulation of NAc DA efflux. For example, it has also been proposed that the CeN could modulate NAc DA efflux via a GABAergic projection to the VTA, which may influence DA neurons (Everitt et al., 1999, 2000). Although efferents from the CeN do terminate in the VTA (Wallace et al., 1992; Fudge and Haber, 2000), evidence that these fibers synapse directly on mesoaccumbens DA neurons is lacking. Alternatively, the CeN may modulate DA cell firing in the VTA indirectly, via local circuit GABAergic interneurons (Wallace et al., 1992). The fact remains that brief stimulation of the CeN in the present study failed to evoke an increase in DA efflux, whereas a significant elevation in DA efflux was evoked by a similar level of activation in the BLA. However, given the 10 min sampling period used here, short acute changes in DA efflux cannot be ruled out.

Recent findings in our laboratory show that reverse dialysis of lido into the CeN significantly decreases basal DA levels in the NAc by  $\sim 20\%$  (Ahn and Phillips, 2001). Thus, GABAergic afferents from the CeN to the VTA may inhibit the extensive GABAergic interneuron network in the VTA under normal conditions. Inactivation of the CeN with lido would in turn increase the activity of inhibitory VTA GABAergic interneurons, thereby suppressing the firing of mesoaccumbens DA cells (Ahn and Phillips, 2001). According to this anatomical arrangement, brief

stimulation of the GABAergic afferents from the CeN to the VTA could cause phasic inhibition of GABAergic interneurons and short-lasting increases in DA cell firing. Clearly, future experiments will be necessary to test these hypotheses.

### Evidence for local modulation of DA efflux in the NAc by the BLA

Stimulation of the ventral subiculum of the hippocampus increases DA efflux in the NAc in both an impulse-dependent and impulse-independent manner (Blaha et al., 1997; Legault et al., 2000; Taepavarapruk and Phillips, 2001). Although the mechanisms by which the BLA can modulate NAc DA efflux are still poorly understood, data from the present experiments as well as Floresco et al. (1998), suggest that high-frequency stimulation of the BLA may regulate DA efflux in an impulse-independent manner. In the present study, only stimulation of the caudal BLA results in long-lasting increases in NAc DA efflux. As mentioned previously, this region of the BLA sends direct glutamatergic afferents to the NAc that overlap anatomically and functionally with mesoaccumbens DA afferents in the NAc. iGluRs are expressed in midbrain DA neurons (Sato et al., 1993; Standaert et al., 1994) and glutamatergic BLA afferents synapse in close apposition to tyrosine hydroxylase-containing varicosities on spines of individual medium spiny neurons (Johnson et al., 1994). Furthermore, NMDA receptors are located on tyrosine hydroxylase-containing varicosities in the NAc shell (Gracy and Pickel, 1996), thus providing a mechanism by which glutamate released from BLA afferents could modulate DA efflux presynaptically. Injections of glutamate or iGluR agonists into the NAc increases NAc DA efflux (Imperato et al., 1990; Desce et al., 1992; Ruzicka and Jhamandas, 1993; Youngren et al., 1993), whereas 6-OHDA lesions of the NAc reduce glutamate receptor binding in the NAc (French et al., 1985; Zavitsanou et al., 1996). Taken together, these findings support the hypothesis that iGluRs in the NAc may modulate DA efflux in an impulse-independent manner and are consistent with the finding that reverse dialysis of antagonists of iGluR into the NAc can block increases in DA efflux observed after BLA stimulation.

Two lines of evidence from the present study argue against an exclusive role for activation of cell firing in the VTA in BLA stimulation-evoked increases in DA efflux in the NAc. First, stimulation of the BLA remained effective at increasing NAc DA efflux even though DA cell body firing in the VTA was significantly suppressed by the infusion of a  $\text{Na}^+$  channel blocker (Fig. 3). A similar result was observed with an anesthetized preparation using chronoamperometry to monitor changes in DA oxidation current (Floresco et al., 1998). Second, inactivation of the mPFC also failed to block the increase in NAc DA efflux after BLA stimulation (Fig. 4). These two experiments provide support for our conjecture that regulation by the BLA of DA efflux in the NAc does not depend on activation of either DA cell bodies in the VTA or projection neurons from the mPFC to the VTA.

Although the caudal BLA does not project directly to the VTA, the BLA could still influence DA cell body firing through other indirect pathways including the direct glutamatergic afferent projection from the BLA to the mPFC (Jackson and Moghaddam, 2001). The mPFC is uniquely positioned to modulate DA efflux in the NAc as it projects to both the VTA and NAc (Kelley et al., 1982; Sesack et al., 1989), and evidence suggests that the mPFC may regulate DA efflux in the NAc through its glutamatergic afferents to the VTA (Taber and Fibiger, 1995; Karreman and Moghaddam, 1996). Additionally, others have

shown that during activation of the BLA, changes in NAc DA efflux may be regulated by BLA afferents to the mPFC (Jackson and Moghaddam, 2001).

Jackson and Moghaddam (2001) reported that DA efflux in the NAc did not change significantly during a 10 min train of BLA stimulation, an effect these authors attributed to increased glutamate transmission in the mPFC. Despite the notable differences in the duration of electrical stimulation of the BLA in the study of Jackson and Moghaddam (2001) (10 min) as compared with the present experiments (10 sec), a significant increase in DA efflux in the NAc was observed in both studies after cessation of BLA stimulation. Interestingly, blockade of glutamate transmission in the mPFC did not affect the long-lasting increase in NAc DA efflux observed after prolonged stimulation of the BLA (Jackson and Moghaddam, 2001). When combined with the similar effect of lido inactivation of the mPFC in the present study, these data provide further support for our conclusion that stimulation of the BLA can modulate NAc DA efflux directly via local mechanisms within the NAc.

### Functional implications

Everitt et al. (1999, 2000) emphasize that theories of amygdala function should account for different roles of amygdala nuclei in controlling adaptive behavioral responses. Specifically, they propose that interactions between the CeN and mesoaccumbens DA system in the VTA are necessary for reflexive Pavlovian conditioned responses, such as autoshaping (Parkinson et al., 2000a) and freezing behavior (Killcross et al., 1997). More flexible operant responses, demonstrated with conditioned reinforcement paradigms, appear to be subserved by interactions between the BLA and NAc (Everitt and Robbins, 1992; Killcross et al., 1997; Everitt et al., 1999; 2000; Parkinson et al., 2000a). The present data show that brief stimulation of the CeN comparable with firing rates elicited by rewarding stimuli (Muramoto et al., 1993; Uwano et al., 1995) does not affect DA efflux in the NAc when integrated over a 10 min period. However, the possibility still remains that activation of the CeN can elicit short-lasting changes in mesoaccumbens DA efflux. In contrast, stimulation of the BLA induces a long-lasting increase in DA efflux in the NAc. Additional data from our laboratory suggest that the BLA can autoregulate its afferent input to the NAc as a consequence of evoked increases in DA efflux (Floresco et al., 2001a). In summary, the interaction of the CeN with the DA cell bodies in the VTA may cause the animal to engage in a more restrictive, reflexive response to salient stimuli, whereas local changes in DA efflux in the NAc produced by high-frequency stimulation of the BLA may facilitate “behavioral switching” in complex, constantly changing environments (Floresco et al., 2001a,b).

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