

# AMPA Receptor Stimulation within the Central Nucleus of the Amygdala Elicits a Differential Activation of Central Dopaminergic Systems

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Appetitive and aversive arousing stimuli increase rates of dopamine (DA) release, particularly within the prefrontal cortex (PFC). Evidence suggests an activating influence of both the central (CeA) and basolateral (BLA) nuclei of the amygdala on DA neurotransmission. For example, lesions of CeA block stressor-induced increases in DA release. Additionally, electrical stimulation of BLA increases DA release in select terminal fields. Previous studies indicate that glutamatergic AMPA receptors modulate CeA and BLA output. However, the extent to which AMPA receptors participate in amygdala-dependent activation of DA neurotransmission is unknown. The current studies examined the effects of bilateral AMPA infusions within CeA or BLA on post-mortem and *in vivo* microdialysis indices of DA release. Additionally, stress is associated with moderate increases in serotonin (5-HT) neurotransmission that are also blocked by CeA lesions. Thus, the current studies also examined the impact of AMPA infusions on post-mortem indices of 5-HT utilization. AMPA infusion into CeA, but not BLA, increased post-mortem indices of DA and 5-HT release in a pattern comparable to that observed under appetitive/aversive conditions. *In vivo* microdialysis studies confirmed that AMPA infusions into CeA, but not BLA, increase extracellular PFC DA levels. When infused into sleeping animals, CeA-AMPA infusion also elicited a rapid-onset transition into waking. Thus, CeA-AMPA receptors exert an excitatory influence on DA and 5-HT neurotransmission and on behavioral state. Combined, these results suggest that CeA-AMPA receptors may participate in the coordination of neural systems involved in the regulation of behavioral state under high-arousal conditions.

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## INTRODUCTION

Central dopaminergic systems have been implicated in a broad array of state-dependent affective and cognitive processes. Dopaminergic neurons project widely throughout the brain and are responsive to salient and arousing sensory stimuli (both appetitive and aversive; Thierry *et al*, 1976; Dunn and Everitt, 1988; Roth *et al*, 1988; Fiorino *et al*, 1997; Bassareo and Di Chiara, 1997; Ahn and Phillips, 1999). A differential sensitivity of dopaminergic efferents to these stimuli is observed across terminal fields: dopamine (DA) efferents projecting to the prefrontal cortex (PFC) display the greatest degree of activation, with lesser magnitude increases in DA release observed within the nucleus

accumbens (NAcc), and lesser still within the striatum (Dunn and Everitt, 1988; Abercrombie *et al*, 1989; Bassareo and Di Chiara, 1997; Berridge *et al*, 1999; Cousins *et al*, 1999).

Both the central (CeA) and basolateral (BLA) nuclei of the amygdala, two structures implicated in aversively and appetitively motivated learning and behavior (Pascoe and Kapp, 1985; Gallagher and Chiba, 1996; Parkinson *et al*, 2000; LeDoux, 2000; Davis, 2000; Cardinal *et al*, 2002), appear to influence rates of DA neurotransmission. For example, lesions of CeA block stressor-induced increases in DA release in PFC and NAcc (Davis *et al*, 1994; Goldstein *et al*, 1996). Recent observations using lidocaine infusions into CeA suggest that CeA also contributes to increases in DA neurotransmission associated with appetitive conditions (Ahn and Phillips, 2003). Although the circuitry underlying CeA-dependent alterations in DA neurotransmission remains unknown, anatomical evidence indicates that CeA provides direct (Gonzales and Chesselet, 1990; Wallace *et al*, 1992) or indirect (Fadel *et al*, 2001; Fadel and Deutch, 2002) input to brainstem dopaminergic nuclei. Additional

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evidence also suggests a prominent role of BLA in the modulation of DA neurotransmission. For example, electrical stimulation of BLA increases DA efflux within NAcc (Floresco *et al*, 1998; Howland *et al*, 2002) and PFC (Jackson and Moghaddam, 2001).

The above-described observations suggest a pivotal role of both CeA and BLA in the modulation of DA neurotransmission, at least under certain environmental conditions. The effects of CeA lesions and lidocaine infusions into CeA demonstrate the *necessity* of this nucleus in the enhancement of DA neurotransmission observed under certain high-arousal environmental conditions. However, the extent to which CeA neuronal activation is *sufficient* to elicit a stress- and/or reward-like activation of cerebral dopaminergic systems remains unclear.

Currently, little is known regarding the neurochemical identity of afferents to CeA or BLA involved in the modulation of DA neurotransmission. However, available evidence indicates a prominent role of glutamatergic receptors within CeA and BLA in the acquisition and expression of conditioned aversive responses (Kim *et al*, 1993; Walker and Davis, 2002, 1997). This suggests the possibility that glutamatergic receptors participate in CeA- and/or BLA-dependent alterations in rates of DA neurotransmission.

To better address these issues, the current studies examined the effects of microinfusion of the glutamatergic agonist AMPA into CeA, BLA, or surrounding regions, on post-mortem and microdialysis indices of DA release within select terminal fields in unanesthetized rats. Additionally, CeA appears to exert a modulatory influence on serotonin (5-HT) utilization in stress. For example, lesions of CeA prevent stressor-induced increases in 5-HT utilization (Goldstein *et al*, 1996). Thus, to assess the extent to which AMPA-induced alterations in rates of DA neurotransmission are specific to DA or are also associated with enhanced rates of 5-HT neurotransmission, the current studies also examined the effects of CeA- and BLA-AMPA infusions on indices of 5-HT utilization.

## MATERIALS AND METHODS

### Animals and Surgery

Male Sprague-Dawley rats (280–350 g; Charles River, Wilmington, MA, USA) were housed in pairs on a 13–11 h cycle (lights on 0600) and provided *ad libitum* access to food and water. Stereotaxic surgery was performed using halothane anesthesia administered through a nose cone, with the skull level and body temperature maintained at approximately 36°C. In all cases, guide cannulae (26 or 23 ga.; Plastics One, Roanoke, VA, USA) were implanted bilaterally aimed at either CeA (−2.3A, ± 4.0L, −7.15V) or BLA (−2.85A, ± 4.8L, −7.6V), or immediately dorsal to CeA (−2.3A, ± 4.0L, −6.7V). For *in vivo* microdialysis studies, a microdialysis probe was implanted within the left ( $n = 11$ ) or right ( $n = 3$ ) PFC (+3.2A, ± 1.0L, −5.0V at 4°). In some cases, a bipolar surface-to-depth EEG electrode was implanted within the contralateral PFC (+3.0A, ± 1.5L, −1.5V), as described previously (Berridge and Foote, 1991, 1996; Berridge and Abercrombie, 1999). Cannulae, microdialysis probes, and EEG electrodes were held in position

with acrylic cement (Plastics One). A stainless-steel wire stylet was inserted and attached to each cannula via a threaded connector (Plastics One). For post-mortem studies, surgery was performed 5–10 days prior to testing. For microdialysis studies, surgery was performed on the day prior to testing (see below). All efforts were made to minimize animal suffering and to minimize the number of animals used. All experiments were conducted in accordance with University of Wisconsin-Madison Institutional Animal Care and Use Committee guidelines, and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*.

### Drug Infusions

Infusions were made using the following procedures. PE20 tubing, filled with water, was connected to the infusion needle and housed within a stainless-steel coil spring. The distal end of the tubing was connected to a 10 µl syringe, the plunger of which was advanced by a microprocessor-controlled infusion pump (Harvard Apparatus, South Natick, MA, USA). An air bubble was placed in the tubing to permit visualization of fluid displacement during advancement of the syringe plunger. The needle and tubing were loaded with approximately 5 µl of vehicle or AMPA hydrobromide (RBI; Natick, MA, USA) dissolved in vehicle (20 ng/µl). Artificial extracellular fluid (AECF) containing 0.5% Pontamine Sky Blue dye served as vehicle. An approximately 50 nl air bubble was used to separate vehicle or AMPA from water contained within the PE20 tubing. AMPA or vehicle was infused bilaterally using a procedure that consisted of two 1-min infusions at 150 or 250 nl/min, separated by 2 min. This infusion procedure was based on results obtained in pilot studies that examined the actions of AMPA on locus coeruleus (LC) neuronal discharge rate, using a previously described infusion/recording probe (Berridge and Foote, 1991). In these studies ( $n = 2$ ), 150 nl of AMPA (20 ng/µl) elicited a robust short-duration increase in neuronal discharge rate when the infusion needle was ~200–400 µm from the recording electrode (average increase over baseline was 516% for 3.5 min, at which point neuronal discharge had returned to baseline levels). Higher AMPA concentrations or larger infusion volumes elicited a brief and robust neuronal activation followed by an abrupt cessation of neuronal discharge activity (ie depolarization block), which partially recovered over 3–4 min. In the range of doses used in these studies (see below), two 1-min infusions separated by 2 min resulted in sustained increases in neuronal activity for 6–7 min, with a subsequent return to baseline levels of activity.

### Experimental Procedures

*Post-mortem studies.* Rats were handled daily and weighed approximately every third day from the time of surgery until testing (8–11 days total). To minimize the stress of the infusion procedure, animals were also habituated to the experimental procedure by exposing them to a simulated infusion regimen for 3 consecutive days ending 2 days prior to testing. On the day of testing, each home-cage was moved to an adjacent room (to avoid disturbing the remaining animals in the colony room) and

bilateral drug or vehicle infusions were made within CeA or BLA. As described above, two 1-min bilateral infusions were made, with a 2-min period separating the two infusions.

Two experiments were conducted. In Experiment 1a, 33 ga. needles were used, and infusions consisted of either 150 nl of vehicle or 3.0 ng AMPA. In Experiment 1b, 30 ga. needles were used, and infusions consisted of either 250 nl vehicle or 5.0 ng AMPA. A larger infusion volume and larger diameter needles were used in Experiment 1b because histological data from Experiment 1a indicated that, in a moderate percentage of cases, infusate either did not enter the tissue or did not diffuse radially from the needle-tip (see Results).

At 30 min following infusions, animals were removed from their cages, decapitated, and the brains removed and dissected rapidly on ice. Dissection was performed with the combined use of a brain mold (ASI, Warren, MI, USA) to generate coronal slices, and free-hand dissection. The following brain regions were collected, using a protocol described previously (Dunn and File, 1983; Berridge *et al*, 1999): left and right medial PFC (LPFC and RPFC), collected from a 2 mm slice starting 2 mm posterior to the anterior-most aspect of the frontal cortex; cingulate cortex, collected from a 2 mm slice starting 1 mm posterior to the PFC slice; NAcc, divided into shell and core subdivisions, taken from the same slice as cingulate cortex; and, striatum, also taken from the same slice as cingulate cortex. Dissected tissue was weighed, frozen on dry ice, and stored at  $-80^{\circ}\text{C}$ .

**In vivo microdialysis studies.** For microdialysis studies, probes (see below) were lowered slowly over a 5–10-min period. This procedure was based on previous studies indicating that slow implantation results in substantially higher extracellular levels of norepinephrine and DA. Following probe implant, animals were placed in a Plexiglas testing chamber ( $32 \times 32 \times 40$  cm) overnight. This chamber was housed in a wooden sound-attenuating outer chamber containing a 15 W light bulb, a speaker through which white noise (80 dB) was played, and a 12 V fan running at reduced speed. A 10-cm hole in the top of the chamber permitted passage of infusion, EEG recording, and microdialysis lines. A second 10-cm hole in the front of the chamber permitted videotaping of the animal. A steel coil spring containing the microdialysis probe inlet and outlet was attached to a liquid swivel (Instech Laboratories Inc., Plymouth Meeting, PA, USA) held in a counter-balance outside the outer chamber. A 3-ml syringe containing AECF was connected to the inlet of the liquid swivel via PE20 tubing, and the outlet of the liquid swivel was connected to the microdialysis probe inlet tubing. The plunger of the syringe was advanced by a microprocessor-controlled infusion pump (Harvard Apparatus, South Natick, MA, USA). Fused silica tubing provided outflow to a sample collection vial outside the sound-attenuating chamber (see below).

On the day following surgery, stylets were removed from the guide cannulae and 30 ga. needles were inserted and secured via plastic threaded sleeves. Animals were then left undisturbed while baseline dialysate samples were collected (16-min collection time). Behavior and, when appropriate, EEG were simultaneously recorded onto videotape. EEG was also recorded onto a polygraph. Following a minimum of

three to four consecutive samples during which the animal mostly slept and minimal variation in DA levels was observed (ie less than 10% variation from the mean level), bilateral drug or vehicle infusions were made within CeA or BLA. In several additional cases, unilateral drug infusions were made within CeA. Given that the infusion pump was outside the testing chamber, animals were not handled or otherwise disturbed by the infusion procedure. Infusions were performed as described for Experiment 1b. Following infusions, dialysate samples were collected until DA levels returned to baseline levels for two consecutive samples. Note that 16-min samples were collected continuously and thus the first post-infusion sample period included the 4-min infusion procedure. In most cases, a final dialysate sample was collected during which the animal was handled repeatedly over a 60-s period to elicit a state of moderate arousal or stress. This procedure permitted a comparison of AMPA-induced alterations in DA release with those elicited by stress or high arousal.

### High-Pressure Liquid Chromatography (HPLC) Analyses

In both post-mortem and microdialysis studies, the samples were analyzed using HPLC with electrochemical detection. Post-mortem samples were homogenized in 0.1 N  $\text{HClO}_4$  containing an internal standard (isoproterenol), centrifuged, and injected onto the HPLC system for analyses of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-HT, and 5-hydroxyindole-acetic acid (5-HIAA), as described previously (Berridge *et al*, 1999). The HPLC system consisted of an ESA 580 pump set at 0.65 ml/min, a C18 column ( $150 \times 3.2$  mm; ESA, Inc., Chelmsford, MA, USA), and an amperometric detector (Waters Corp., Milford, MA, USA) with the potential set to  $+0.65$  V. The output of the detector was sent to a computer running analytical software (EZChrom, Scientific Software, Inc., San Ramon, CA, USA). Mobile phase consisted of 0.05 M  $\text{NaH}_2\text{PO}_4$ , 0.05 M sodium citrate, 100  $\mu\text{M}$  EDTA, 9% v/v methanol, and 0.15–0.25 mM octyl sodium sulfate, at pH 2.8–3.4. All reagents were obtained from Fisher Scientific (Itasca, IL, USA) except for octyl sodium sulfate (Sigma Chemical Co., St Louis, MO, USA). Concentrations of DA, DOPAC, 5-HT, and 5-HIAA in each region were calculated from the combined use of external and internal standards and expressed as pg per mg wet tissue. Ratios of DOPAC/DA and 5-HIAA/5-HT were calculated as an index of DA and 5-HT release, respectively.

For *in vivo* microdialysis studies, DA levels were measured using previously described methods (Abercrombie *et al*, 1989; Berridge and Abercrombie, 1999). Briefly, AECF was delivered at a rate of 1.5  $\mu\text{l}/\text{min}$  through PE10 tubing to a length of Spectra/Por hollow dialysis membrane (MW cutoff 6000 Da, o.d. 250  $\mu\text{m}$ ). The membrane was sealed and attached to the PE10 tubing with epoxy. The length of functional dialysis membrane in contact with tissue was 4 mm. A 75-cm length of fused silica (150  $\mu\text{m}$  o.d., 75  $\mu\text{m}$  i.d.) provided outflow. Aliquots of 20  $\mu\text{l}$  were injected onto an HPLC system consisting of an ESA 580 pump set at 0.65 ml/min, a Velosep C18  $100 \times 3.2$  mm cartridge column, and an ESA Model 5200A detector with two electrodes in series:  $-25$  mV,  $+220$  mV. The mobile

phase consisted of 200 mM sodium phosphate, 100  $\mu$ M EDTA, 0.30–0.38 mM octyl sodium sulfate, and 5% v/v methanol, at a pH of 4.85. The quantitation limit for DA (using a criterion of three times background noise) was approximately 0.3 pg.

### EEG Recordings

A bipolar surface-to-depth electrode was used to record cortical EEG, as described previously (Berridge and Foote, 1996). A screw electrode was placed over the cerebellum and served as ground. The ends of the EEG and screw electrodes were inserted into a five-pin plastic connector. EEG signals were amplified, filtered (0.3–100.0 Hz), and recorded on a polygraph and VCR recording tape.

### Blood Collection and Corticosterone (CORT) Assay

For Experiment 1b, trunk blood was collected in heparinized tubes and stored temporarily on ice. Plasma was separated by centrifuge within 5 h and frozen at  $-80^{\circ}\text{C}$ . CORT was measured using a radioimmunoassay kit (Coat-a-Count Rat Corticosterone, Diagnostic Products Corporation, Los Angeles, CA, USA). All samples and standards were assayed in duplicate and the average calculated.

### Histological Assessment of Infusion Needle and Dialysis Probe Placement

Following experimental testing, each animal was deeply anesthetized and perfused transcardially with 4% formaldehyde solution, and the brain was removed, stored in 4% formaldehyde for 48 h, frozen, and sliced into 50  $\mu$ m sections through the infusion site and, when appropriate, the microdialysis probe implant site. Tissue sections were stained with the Neutral Red dye for subsequent examination of these sites. The presence and extent of the spread of Pontamine Sky Blue dye at the infusion site in freshly sectioned tissue was noted to determine: (i) whether fluid exited the needle; and (ii) the location of the center of the infusion site. The latter was determined primarily to note cases in which the majority of infusate traveled dorsally along the infusion needle, rather than radially from the tip of the infusion needle.

### Data Selection Criteria

Data were included in the analyses only when: (1) histological analyses showed accurate placement of infusion needles, that the infusate entered tissue, and that the majority of the infusate did not travel dorsally along the needle; (2) histological analyses showed accurate placement of dialysis probes; and (3) DA concentrations were stable ( $\pm 10\%$  of the mean) throughout the baseline portion of the experiment.

### Statistical Analyses

For post-mortem experiments, neurochemical data from each brain region were analyzed using a one-way ANOVA, with two levels of treatment (CeA-AMPA and CeA-vehicle) for Experiment 1a, and three levels of treatment (CeA-

AMPA, CeA-vehicle, and BIA-AMPA) for Experiment 1b. Right and left PFC data from each experiment were analyzed using a mixed within (hemisphere)/between (treatment)-subjects ANOVA. When statistical significance ( $p < 0.05$ ) was indicated, *post hoc* analyses were conducted using Tukey's HSD test. *In vivo* microdialysis data were analyzed using a mixed within (time/sample #)/between (treatment)-subjects ANOVA. When statistical significance ( $p < 0.05$ ) was indicated, *post hoc* analyses were conducted using Tukey's HSD test. SPSS 11.0.1 (Chicago, IL, USA) was used for all statistical analyses.

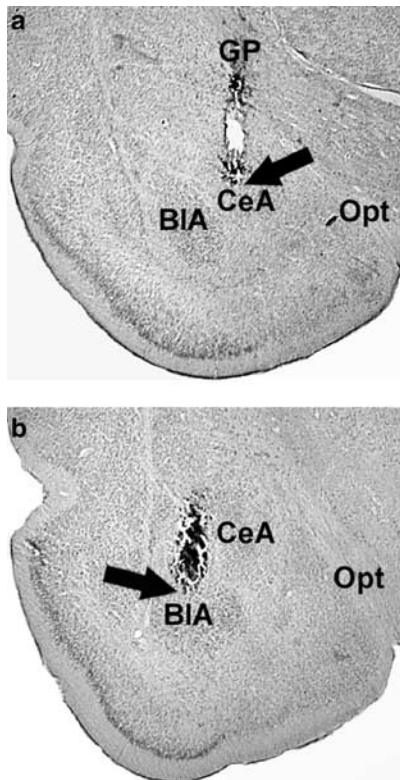
## RESULTS

### Experiment 1. Effects of AMPA Infusions within CeA or BIA on Post-Mortem Indices of DA and 5-HT Release

**General observations.** To assess the extent to which AMPA receptor activation within CeA alters DA and/or 5-HT utilization, tissue levels of DA, DOPAC, 5-HT, and 5-HIAA were measured in six brain regions collected 30 min after AMPA or vehicle infusions. Figure 1a shows a photomicrograph of a typical infusion site. In Experiment 1a, AMPA was administered at a dose of 3.0 ng/150 nl  $\times$  2 infusions/hemisphere (total AMPA/hemisphere = 6.0 ng) using 33 ga. infusion needles (see Methods). Histological data indicated that these infusion parameters occasionally resulted in an inadequate spread of infusate radially from the infusion needle tip (in approximately 35% of cases). In some cases the majority of dye was located dorsal to the tip of the infusion needle, while in other cases, there was a complete absence of dye within the tissue, indicating a failure of infusate to enter the tissue. Previous electrophysiological studies using these infusion volumes and this diameter infusion needle indicate that when either an absence of dye is observed, or the majority of dye is located dorsal to the infusion needle tip, neuronal activity is not altered in the vicinity of the infusion needle tip (Berridge and Foote, 1991; Berridge *et al*, 1993). Thus, in these cases, animals were excluded from the analyses. In an attempt to render a higher percentage of usable cases, a slightly different set of infusion parameters was chosen for Experiment 1b: infusion volume was 250 nl (5.0 ng/250 nl  $\times$  2 infusions/hemisphere, total AMPA/hemisphere = 10 ng) and 30 ga. infusion needles were used. Experiment 1b also included a group in which AMPA was infused into BIA (Figure 1b) to assess preliminarily whether BIA-AMPA infusions impact DA and/or 5-HT neurotransmission similar to CeA-AMPA infusions (also see Experiment 2).

In both Experiments 1a and 1b, histological data revealed that some animals that received vehicle infusions within CeA did not have dye present in at least one hemisphere (Experiment 1a,  $n = 5$ ; Experiment 1b,  $n = 4$ ). Statistical analysis revealed that these animals did not differ from vehicle-treated animals with dye present in both hemispheres. For this reason, and because these cases nonetheless provided a control for needle insertion, handling, and infusion procedures, all animals receiving vehicle infusions were included in the analyses.

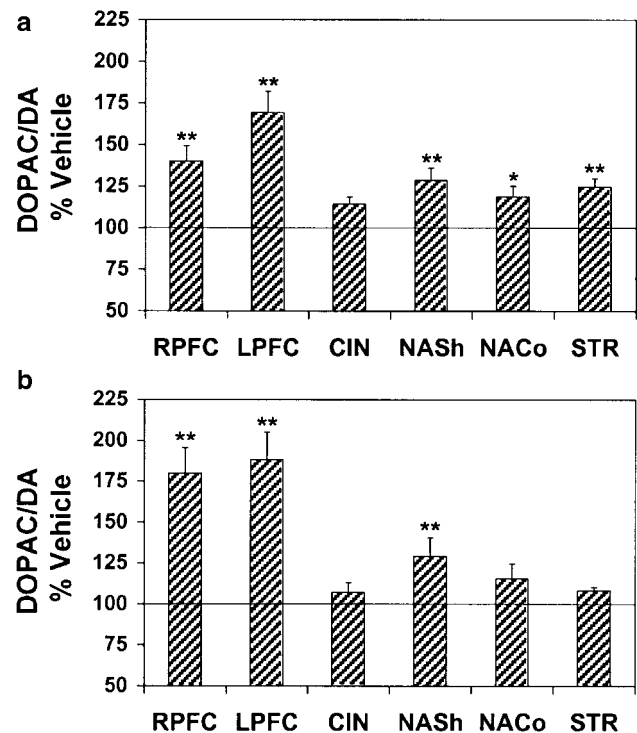
In both Experiments 1a and 1b, the majority of animals were asleep immediately prior to transfer to the infusion chamber. All animals were awake at the time of the



**Figure 1** Photomicrographs of CeA and BIA infusion sites. Shown are CeA (a) and BIA (b) infusion sites in Neutral Red-stained coronal sections. Arrows indicate the most ventral extent of each infusion needle track. CeA, central nucleus of the amygdala; BIA, basolateral nucleus of the amygdala; GP, globus pallidus; Opt, optic tract.

infusions due to the infusion procedures (see Methods). Under these conditions, AMPA infusions had no obvious effect on behavior, except perhaps a slight increase in freezing behavior in the CeA-AMPA groups. Abnormal behavior (eg seizure-like) was not observed at any time during or following the infusions. The majority of animals had returned to sleep 30 min following the infusions, immediately prior to tissue collection.

**Effects of AMPA infusions within CeA on DOPAC/DA and 5-HIAA/5-HT ratios.** The effects of AMPA infusions within CeA on DOPAC/DA ratios across various brain regions are depicted in Figure 2 (see Table 1 for absolute DA and DOPAC concentrations). Across brain regions, bilateral infusions of AMPA within CeA elicited an activation of DA and 5-HT systems similar to that observed in stress (Dunn and Everitt, 1988; Abercrombie *et al*, 1989; Bassareo and Di Chiara, 1997; Berridge *et al*, 1999). Bilateral AMPA infusions within CeA elicited a significant increase in PFC DOPAC/DA levels (Experiment 1a: 55% increase,  $F_{1,14} = 28.5$ ,  $p < 0.001$ ; Experiment 1b: 84% increase,  $F_{2,17} = 38.0$ ,  $p < 0.001$ ). Previous studies have indicated a possible hemispheric asymmetry in PFC DA release in stress (Berridge *et al*, 1999). Therefore, in the current studies, left and right PFC hemispheres were analyzed separately. In neither Experiment 1a nor 1b was a significant effect of hemisphere observed within PFC. However, in Experiment 1a, a significant hemisphere  $\times$  treatment interaction was observed ( $F_{1,14} = 6.5$ ,  $p = 0.024$ ), with a greater increase in



**Figure 2** Effects of bilateral AMPA infusion within CeA on DOPAC/DA in right and left prefrontal cortex (RPFC and LPFC), cingulate cortex (CIN), nucleus accumbens shell (NASH), nucleus accumbens core (NACo), and striatum (STR). Top panel (a) displays results from Experiment 1a, in which infusions consisted of 3 ng AMPA per hemisphere. Bottom panel (b) displays results from Experiment 1b, in which infusions consisted of 5 ng AMPA per hemisphere. Shown are mean ( $\pm$  SEM) DOPAC/DA ratios expressed as a percentage of vehicle-treated controls. In both studies, bilateral AMPA infusion within CeA increased DOPAC/DA ratios within LPFC, RPFC, and NASH. Less consistent increases in DOPAC/DA were seen in NACo and STR. \* $p < 0.05$ , \*\* $p < 0.01$  vs vehicle controls.

DOPAC/DA observed in LPFC in the CeA-AMPA group (LPFC, 69% increase; RPFC, 40% increase). In contrast, there was no significant hemisphere  $\times$  treatment interaction observed in Experiment 1b ( $F_{2,17} = 0.3$ ,  $p = 0.76$ , LPFC, 88% increase, RPFC, 80% increase).

AMPA infusions elicited smaller, although significant, increases in DOPAC/DA within NAcc shell relative to those observed in PFC (Experiment 1a: 29% increase,  $F_{1,14} = 13.0$ ,  $p = 0.003$ ; Experiment 1b: 29% increase,  $F_{2,18} = 5.4$ ,  $p = 0.014$ ). Although a trend for increased DOPAC/DA was observed within the NAcc core, this was statistically significant in Experiment 1a only (Experiment 1a: 19% increase,  $F_{1,14} = 5.5$ ,  $p = 0.034$ ; Experiment 1b: 16% increase  $F_{2,18} = 2.1$ ,  $p = 0.150$ ). Similarly, a small increase in DOPAC/DA was observed within striatum, which was significant in Experiment 1a (24% increase,  $F_{1,14} = 9.5$ ,  $p = 0.008$ ) but not Experiment 1b (8% increase,  $F_{2,18} = 1.7$ ,  $p = 0.22$ ). AMPA infusions tended to elicit marginal or insignificant increases in DOPAC/DA within the cingulate cortex (Experiment 1a: 14% increase,  $F_{1,14} = 4.6$ ,  $p = 0.05$ ; Experiment 1b: 7% increase,  $F_{2,18} = 1.7$ ,  $p = 0.21$ ).

To determine the extent to which the above-described effects of AMPA infusions into CeA are specific to DA, the effects of these infusions on 5-HT utilization were also assessed. As shown in Figure 3, bilateral AMPA infusions

**Table 1** Picogram DA, DOPAC, 5-HT, and 5-HIAA per mg Wet Tissue, and DOPAC/DA and 5-HIAA/5-HT Ratios of CeA-Vehicle and CeA-AMPA Groups

	RPFC	LPFC	CIN	NASh	NACo	STR
<i>Vehicle</i>						
DA	116.5 ± 9.3	129.6 ± 9.8	185.1 ± 27.7	12432.4 ± 725.7	14211.2 ± 1148.5	18060.2 ± 1003.1
DOPAC	31.2 ± 2.8	31.5 ± 3.1	66.6 ± 7.2	1865.6 ± 137.7	2443.4 ± 228.6	1863.7 ± 102.7
DOPAC/DA	0.27 ± 0.01	0.24 ± 0.01	0.37 ± 0.02	0.15 ± 0.01	0.17 ± 0.01	0.11 ± 0.01
<i>AMPA</i>						
DA	112.0 ± 6.9	104.0 ± 8.2	195.2 ± 18.8	11336.8 ± 891.9	14582.3 ± 459.3	20107.4 ± 1206.0
DOPAC	41.6 ± 3.2*	41.9 ± 3.8*	81.2 ± 5.7	2164.4 ± 171.2	2936.2 ± 120.0	2629.9 ± 169.3**
DOPAC/DA	0.37 ± 0.03**	0.41 ± 0.03**	0.42 ± 0.02	0.19 ± 0.01**	0.20 ± 0.01*	0.13 ± 0.01**
<i>Vehicle</i>						
5-HT	1925.0 ± 116.0	2260.5 ± 83.0	899.3 ± 54.7	3593.7 ± 155.0	1580.8 ± 108.3	953.6 ± 46.0
5-HIAA	338.1 ± 20.5	382.3 ± 17.4	294.6 ± 20.5	695.8 ± 23.2	712.4 ± 33.5	653.9 ± 27.7
5-HIAA/5-HT	0.18 ± 0.004	0.17 ± 0.01	0.33 ± 0.02	0.20 ± 0.01	0.47 ± 0.03	0.70 ± 0.04
<i>AMPA</i>						
5-HT	2149.3 ± 123.7	2212.3 ± 103.3	906.4 ± 70.2	3915.2 ± 570.1	2609.5 ± 976.2	885.0 ± 44.7
5-HIAA	460.4 ± 26.6**	485.0 ± 20.9**	324.1 ± 24.4	789.3 ± 34.8*	836.2 ± 28.1*	683.0 ± 39.4
5-HIAA/5-HT	0.22 ± 0.01**	0.22 ± 0.01**	0.36 ± 0.03	0.24 ± 0.01**	0.51 ± 0.02	0.77 ± 0.01

Shown are mean ± SEM concentrations of DA, DOPAC, 5-HT, and 5-HIAA, as well as DOPAC/DA and 5-HIAA/5-HT ratios in right and left prefrontal cortex (RPFC and LPFC), cingulate cortex (CIN), nucleus accumbens shell and core (NASh and NACo), and dorsal striatum (STR), from CeA-vehicle and CeA-AMPA groups from Experiment 1a (see Figures 2a and 3a). Data are expressed as pg/mg wet weight of tissue. \* $p < 0.05$ , \*\* $p < 0.01$  vs vehicle controls.

into CeA elicited a relatively small increase in 5-HIAA/5-HT within PFC (Experiment 1a: 26% increase,  $F_{1,14} = 26.4$ ,  $p < 0.001$ ; Experiment 1b: 13% increase,  $F_{2,16} = 1.3$ ,  $p = 0.31$ ). A more consistent increase in 5-HIAA/5-HT was observed within the NAcc shell (Experiment 1a: 22% increase,  $F_{1,14} = 13.4$ ,  $p = 0.003$ ; Experiment 1b: 42% increase,  $F_{2,17} = 7.4$ ,  $p = 0.005$ ). AMPA infusions into CeA did not significantly alter indices of 5-HT utilization within any other region examined in either experiment.

**Effects of AMPA infusions within BLA on DOPAC/DA and 5-HIAA/5-HT ratios.** As described above, Experiment 1b included an additional treatment group in which AMPA was infused bilaterally within BLA to provide a preliminary assessment of the extent to which AMPA-induced neuronal activation within BLA alters indices of either DA or 5-HT utilization (also see Experiment 2). As shown in Figure 4, BLA-AMPA infusions did not appear to alter indices of either DA or 5-HT utilization in any brain region examined. Thus, for those regions in which the overall ANOVA indicated a significant effect of treatment on DOPAC/DA (PFC and NAcc shell; see above for  $F$  and  $p$  values), DOPAC/DA ratios did not differ significantly between BLA-AMPA-treated animals and CeA-vehicle-treated animals (LPFC,  $p = 0.973$ ; RPFC,  $p = 0.751$ ; NAcc shell,  $p = 0.932$ ). Moreover, DOPAC/DA ratios were significantly higher in CeA-AMPA-treated animals than in BLA-AMPA-treated animals (LPFC,  $p < 0.001$ ; RPFC,  $p < 0.001$ ; NAcc shell,  $p = 0.023$ ).

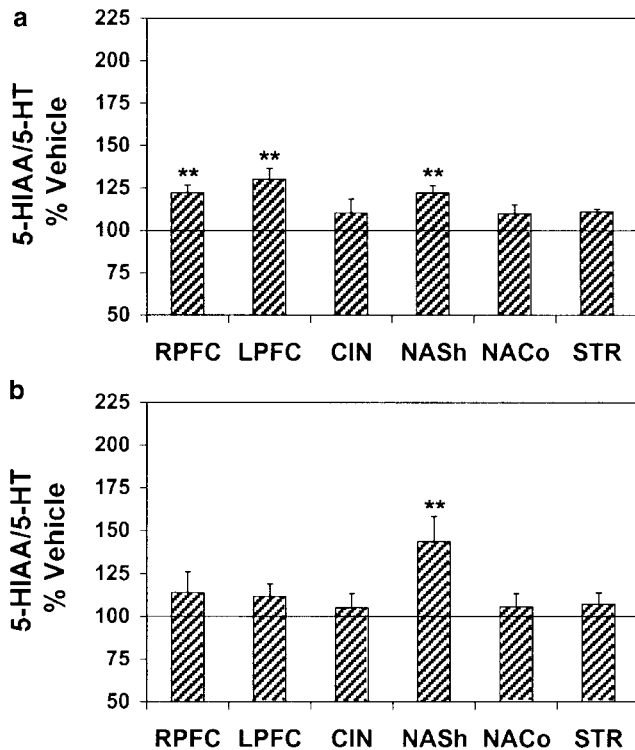
Similarly, also shown in Figure 4, BLA-AMPA infusions did not impact 5-HIAA/5-HT ratios. Thus, in the only brain region in which there was a significant effect of treatment

on 5-HIAA/5-HT (NAcc shell, see above for  $F$  and  $p$  values), 5-HIAA/5-HT ratios did not differ significantly between BLA-AMPA-treated animals and CeA-vehicle-treated animals ( $p = 0.964$ ), whereas CeA-AMPA-treated animals displayed significantly greater 5-HIAA/5-HT ratios than BLA-AMPA-treated animals ( $p = 0.017$ ).

**Effects of AMPA infusions within CeA or BLA on plasma CORT.** Plasma CORT levels were measured in animals 30 min after infusions were made (Experiment 1b only). In the CeA-vehicle group, CORT was  $104.9 \pm 10.4$  ng/ml. AMPA infusions within CeA elicited a large ( $162.7 \pm 30.7$  ng/ml; 155%), although nonsignificant, increase in CORT levels, relative to CeA-vehicle-treated animals ( $F_{2,19} = 2.4$ ,  $p = 0.12$ ). AMPA infusions within BLA did not appear to influence CORT levels ( $115.3 \pm 14.3$  ng/ml).

## Experiment 2. Effects of AMPA Infusions within CeA or BLA on *In Vivo* Microdialysis Measures of PFC DA Release

To confirm results obtained with post-mortem measures and to better characterize the time course of AMPA-induced alterations in rates of DA release, the effects of AMPA infusions into CeA or BLA on extracellular levels of DA in PFC were assessed using *in vivo* microdialysis. These studies utilized the same infusion protocol used in Experiment 1b, except infusions were controlled remotely and performed at a time when animals were asleep. Previous work indicates an arousal-enhancing action of

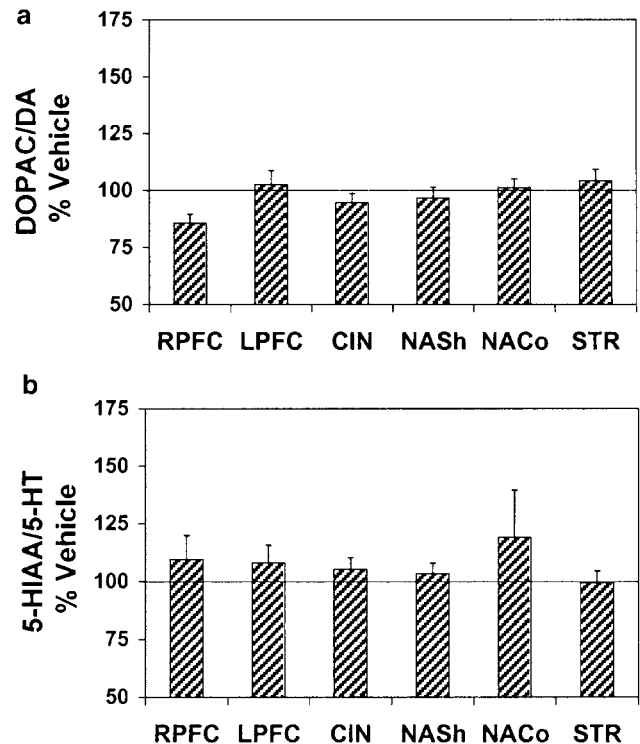


**Figure 3** Effects of bilateral AMPA infusion within CeA on 5-HIAA/5-HT in RPFC and LPFC, CIN, NASH, NACo, and STR. Shown are results from Experiment 1a (a) and Experiment 1b (b). Shown are mean ( $\pm$  SEM) 5-HIAA/5-HT ratios expressed as a percentage of vehicle-treated controls. Bilateral AMPA infusion within CeA consistently increased 5-HIAA/5-HT ratios within NASH and less consistently increased 5-HIAA/5-HT ratios within RPFC and LPFC. \* $p < 0.05$ , \*\* $p < 0.01$  vs vehicle controls.

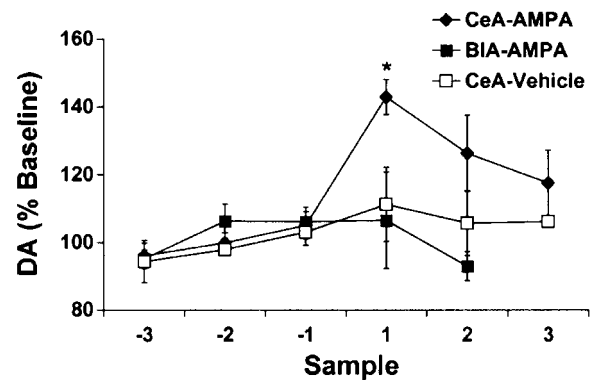
CeA activation (Kapp *et al*, 1994). The extent to which CeA-AMPA receptor activation enhances arousal has not been examined. As a preliminary exploration of this issue and to assess the relationship between AMPA-induced increases in DA release and arousal, behavioral indices of sleep and waking were recorded in all animals throughout the experiment. In a subset of these animals, EEG was also recorded. As in Experiment 1, AMPA infusions into CeA, BIA, or adjacent regions did not elicit atypical behavior.

**Effects of AMPA infusions into CeA or BIA on extracellular DA levels within PFC.** Figure 5 displays the effects of AMPA infusions into CeA and BIA on extracellular DA levels within PFC. Baseline averages of extracellular DA were  $0.83 \pm 0.10$  pg/20  $\mu$ l dialysate for the vehicle group,  $0.70 \pm 0.06$  pg/20  $\mu$ l dialysate for the CeA-AMPA group, and  $0.87 \pm 0.23$  pg/20  $\mu$ l dialysate for the BIA-AMPA group. Infusions were performed during the first 4 min of the first 16-min post-infusion sample. Statistical analyses revealed a significant effect of treatment ( $F_{2,17} = 3.7$ ,  $p = 0.048$ ), but not of time ( $F_{5,85} = 2.0$ ,  $p = 0.08$ ) or treatment  $\times$  time ( $F_{10,85} = 1.3$ ,  $p = 0.25$ ). *Post hoc* analyses indicated that the CeA-AMPA group differed from the CeA-vehicle group only at the first post-infusion time point ( $p = 0.03$ ), when CeA-AMPA DA levels were 143% of baseline. The BIA-AMPA group did not differ from the CeA-vehicle group at any time point.

Within CeA-AMPA-treated animals, left and right PFC DA responses appeared comparable (data not shown).



**Figure 4** Effects of bilateral AMPA infusion within BIA on DOPAC/DA (a) and 5-HIAA/5-HT (b) in RPFC and LPFC, CIN, NASH, NACo, and STR. Data are from Experiment 1b, in which 5 ng AMPA was infused per hemisphere. Shown are mean ( $\pm$  SEM) DOPAC/DA or 5-HIAA/5-HT ratios expressed as a percentage of CeA-vehicle-treated controls. In contrast to results observed with infusion within CeA, bilateral AMPA infusion within BIA did not elicit consistent alterations in either DOPAC/DA or 5-HIAA/5-HT within any region examined. \* $p < 0.05$ , \*\* $p < 0.01$  vs vehicle controls.



**Figure 5** Effects of bilateral infusion of vehicle or AMPA within CeA or bilateral AMPA infusion within BIA on extracellular DA levels as measured by *in vivo* microdialysis. Shown are mean ( $\pm$  SEM) expressed as a percentage of baseline levels for consecutive 16-min samples. Infusions were made immediately following sample #-1, during the first 4 min of sample #1. Bilateral AMPA infusion within CeA, but not BIA, elicited a substantial increase in DA levels within PFC during the first post-infusion sample. \* $p < 0.05$  vs CeA-vehicle.

Within this group, the maximal increase in extracellular DA was generally confined to the first post-infusion sample. Lower levels that remained elevated compared to baseline levels were observed in the second post-infusion sample in approximately 50% of cases. This relatively short-lived

increase in DA levels is consistent with electrophysiological observations in pilot studies described above (see Methods). However, in a limited number of cases ( $n=4$ ), near-maximal DA levels were sustained beyond the first post-infusion sample into post-infusion samples 3 or 4. In these cases, animals also displayed a longer behavioral response (waking) to the AMPA infusions.

Following the return of extracellular DA levels to baseline values, most animals were picked up repeatedly over a 60-s period to elicit a relatively high-arousal state (ie handling-stress). This manipulation resulted in a robust increase in PFC extracellular DA levels ( $n=20$ , mean = 148% of baseline). This magnitude increase in DA levels within PFC is comparable to that described previously with other stressors (Abercrombie *et al*, 1989).

*Effects of AMPA infusions within CeA or BLA on behavioral and EEG measures of sleep and waking.* Behavior was videotaped in all animals used in the microdialysis studies. All animals appeared asleep on the basis of behavioral measures at the time of the infusion (ie body and head on the floor). Following bilateral CeA-AMPA infusions, 11 out of 13 animals woke up (head raised off the floor) within the first 4 min following the start of the infusions (approximately corresponding to the most robust period of neuronal activation as determined in pilot studies; see Methods). Latency to waking was  $102 \pm 20$  s (range = 22–225 s) with an average duration of  $526 \pm 69$  s. In contrast to AMPA-treated animals, only one out of four vehicle-treated animals displayed a brief bout of waking in the same period of time following the start of the infusions (latency = 58 s, duration = 18 s).

A subset of animals was implanted with a cortical EEG electrode ( $n=5$ ) to better characterize the time course of the wake-promoting actions of CeA-AMPA infusions. At the start of the infusion, all animals displayed large-amplitude, low-frequency activity indicative of slow-wave sleep (see Figure 6). In all cases, AMPA infusions within CeA elicited a rapid activation of cortical EEG characterized by the appearance of low-voltage, high-frequency activity. AMPA-induced EEG activation occurred with a mean latency of  $75 \pm 31$  s (range = 31–195) from the beginning of the first 60-s infusion (see Figure 6) and a duration of  $820 \pm 100$  s (range = 600–1078). In contrast, although EEG activation was observed in some of the vehicle-treated animals, it occurred with a substantially longer latency ( $166 \pm 60$ , range = 56–321 s) and a substantially shorter duration ( $77 \pm 43$  s, range = 28–204 s) than that observed in AMPA-treated animals. Interestingly, although BLA-AMPA infusions did not elicit an increase in DA efflux, these infusions ( $n=3$ ) did appear to elicit a moderate increase in waking based on either behavioral (latency =  $134 \pm 47$ , duration =  $68 \pm 22$ ) or EEG measures (latency =  $42 \pm 10$ , duration =  $348 \pm 30$ ).

#### Effects of Unilateral AMPA Infusions within CeA or Bilateral AMPA Infusions Dorsal to CeA on Indices of DA Release

In some cases in Experiments 1a and 1b, histological data indicated that inaccurate needle-tip placement or inade-



**Figure 6** Effects of bilateral infusion of AMPA within CeA on cortical EEG. The dose of AMPA was 5.0 ng/infusion/hemisphere. Shown is a 5-min EEG trace before and during infusions. Bars denote time of infusions. Prior to the infusions, the animal spent the majority of time in slow-wave sleep, characterized by large-amplitude, slow-wave EEG activity. CeA-AMPA infusion elicited a short-latency desynchronization of EEG that persisted, in this case, for approximately 10 min.

quate spread of infusate radially from the infusion needle-tip occurred in one or both hemispheres. In one case from Experiment 1a, bilateral AMPA infusions were made within the globus pallidus, dorsal to CeA. In another case, from Experiment 1b, AMPA infusions were made within the substantia innominata immediately dorsal to CeA in the left hemisphere and within CeA in the right hemisphere. In both of these cases, DOPAC/DA ratios in all brain regions in which they were measured were well within the range of vehicle-infused animals. In addition, in Experiment 2, bilateral AMPA infusions immediately dorsal to CeA were intentionally made in two cases. In neither of these cases did PFC DA levels increase above baseline levels. Combined, these observations indicate that neither infusions dorsal to CeA nor infusions lateral to CeA (ie within BLA) alter indices of DA utilization.

There were also several cases in Experiment 1b in which unilateral AMPA infusions were made within CeA, and an absence of dye was observed within the contralateral hemisphere (unilateral left CeA-AMPA,  $n=5$ ; unilateral right CeA-AMPA,  $n=3$ ). Analyses of these cases indicated that neither left unilateral nor right unilateral CeA-AMPA infusions resulted in DOPAC/DA or 5-HIAA/5-HT ratios outside of the range of vehicle-treated animals (data not shown). In addition, in several cases in Experiment 2, unilateral AMPA infusions within CeA (right CeA  $n=2$ , left CeA  $n=3$ ) were made intentionally. In none of these cases were DA levels altered from baseline.

## DISCUSSION

The current studies demonstrate that stimulation of AMPA receptors within CeA results in a differential activation of dopaminergic efferents across cortical and subcortical terminal fields. Thus, AMPA infusions within CeA elicited a relatively large increase in DA utilization within PFC, smaller increases within the NAcc shell, and even smaller and less consistent increases within the NAcc core and dorsal striatum. This pattern of activation of DA efferents is similar to that observed in high-arousal states such as stress and reward (Dunn and Everitt, 1988; Abercrombie *et al*, 1989; Bassareo and Di Chiara, 1997; Berridge *et al*, 1999; Cousins *et al*, 1999). Activation of CeA-AMPA receptors also elicited smaller and stress-like (Berridge *et al*, 1999) increases in 5-HT utilization in PFC and accumbens shell as well as an increase in plasma CORT levels. When infusions were made into sleeping animals, the AMPA-induced



activation of DA and 5-HT systems was accompanied by a rapid transition from the sleeping to the waking state. Importantly, infusions outside of CeA, including within BLA, did not alter indices of DA or 5-HT neurotransmission. The current results extend previous observations indicating that lesions of CeA suppress stressor-induced activation of DA and 5-HT neurotransmitter systems (Davis *et al*, 1994; Goldstein *et al*, 1996) by demonstrating that (1) increased CeA neuronal activity is sufficient to elicit stress- and/or reward-like activation of these two neurotransmitter systems and, more specifically (2) CeA-AMPA receptors may play a role in these actions. Combined, these observations suggest a critical role of CeA in the regulation of DA and 5-HT neurotransmission in moderate-to-high arousal conditions.

### Methodological Considerations

The current studies used both post-mortem- and microdialysis-based measures of DA neurotransmission. Post-mortem measures permit simultaneous examination of multiple DA and 5-HT terminal fields. Microdialysis permits the verification of alterations in extracellular levels of a given neurotransmitter as well as characterization of the time course of these alterations within a limited set of terminal fields. Post-mortem measures based on DOPAC/DA ratios reduce variability resulting from individual differences in tissue levels of DA and have been demonstrated to reflect alterations in DA neuronal discharge rates (Elchisak *et al*, 1976; Roth *et al*, 1976; Wood and Altar, 1988). Importantly, post-mortem measures have been demonstrated to provide results similar to those obtained with *in vivo* microdialysis-based measures of rates of DA neurotransmission in stress (Dunn and Everitt, 1988; Roth *et al*, 1988; Abercrombie *et al*, 1989). The only conditions under which DOPAC/DA ratios do not accurately reflect alterations in DA release occur following pharmacological manipulations that bypass normal physiological mechanisms regulating neurotransmitter release and metabolism (eg amphetamine, uptake blockers, MAO inhibitors; Roth *et al*, 1973, 1976; Kuczenski, 1983). Finally, results obtained in the current microdialysis studies (Experiment 2) provide evidence that the increases in DOPAC/DA ratios observed in the current post-mortem experiments (Experiment 1a/1b) reflect increases in extracellular DA levels.

The current studies utilized chemical stimulation to examine the consequences of acute neuronal activation within CeA and BLA on rates of DA neurotransmission. An advantage of this approach, as opposed to electrical stimulation, is that it avoids activation of fibers of passage and is dependent on physiologically relevant, receptor-mediated processes. In pilot studies, local AMPA infusions, using similar infusion parameters, resulted in a sustained increase in LC neuronal activity when the infusion needle was placed ~200–400  $\mu$ m from the recording electrode. In these pilot studies, neuronal activity returned to baseline discharge levels following the infusion, and neuronal activation was elicited with repeated infusions (see Methods for further description). Combined, these results provide evidence that, at the dose and volume used in the current studies, AMPA infusions increased neuronal activity within the immediate vicinity of the infusion needle without

eliciting an over-activation of neuronal discharge rate (ie depolarization block) or damaging neurons in the vicinity of the infusion needle (ie excitotoxicity).

### CeA and Arousal

Previous work has demonstrated increases in EEG indices of arousal following electrical stimulation of the CeA (Kapp *et al*, 1994). The current findings demonstrate a similar activation of forebrain EEG following AMPA-induced activation of CeA. Additionally, these studies demonstrate that increases in DA release observed following CeA-AMPA infusions follow a time course that is largely similar to that observed for CeA-dependent activation of forebrain EEG. Available evidence suggests that activation of dopaminergic systems may be associated with waking (Monti *et al*, 1989, 1990; Trampus *et al*, 1991, 1993). Combined, these observations suggest a potential role of CeA-dependent activation of DA systems in the arousal-modulating actions of CeA.

Both 5-HT and acetylcholine have been implicated in the regulation of EEG activity state (Vanderwolf and Robinson, 1981; Jacobs *et al*, 1990; Kapp *et al*, 1994). The current studies demonstrate increased rates of 5-HT release following AMPA infusions into CeA. Additionally, previous work demonstrates that activation of EEG following electrical stimulation of CeA is blocked by cholinergic antagonists (Kapp *et al*, 1994). Thus, these observations suggest the CeA coordinates the activation of multiple ascending neuronal systems that participate in the regulation of behavioral state. Related to this, available evidence suggests CeA may modulate LC discharge activity (Van Bockstaele *et al*, 1998). Moreover, the LC-noradrenergic system has been demonstrated to exert wake-promoting actions (Berridge and Foote, 1991, 1996; Berridge *et al*, 1996). Thus, it might be predicted that AMPA-induced activation of CeA will also elicit an activation of the LC-noradrenergic system.

Given that rates of DA neurotransmission may be state-dependent, it is possible that increased rates of DA release elicited by AMPA infusions are an indirect consequence of the induction of the waking or aroused state. Arguing against this hypothesis are two observations. First, in the studies utilizing post-mortem measures, all infusions were performed in animals that were awake at the time of the infusion, and the majority of animals, regardless of treatment condition, were asleep immediately prior to tissue collection. Thus, under these conditions AMPA infusions did not change the sleep/wake state of the animal relative to vehicle-treated animals. Nonetheless, in these studies, CeA-AMPA infusions resulted in a substantial increase in indices of DA neurotransmission. Second, in the above-described studies utilizing *in vivo* microdialysis, bilateral CeA-AMPA infusions elicited quiet, and not active, waking. Previous studies demonstrate that quiet waking is associated with a relatively small increase in DA levels within PFC (eg approximately 25%; Berridge and Stalnaker, 2002), well below the magnitude of the DA response observed following AMPA infusions within CeA in the current studies.

### Site of Action

The current studies suggest CeA is the critical site of action for the AMPA-induced neurochemical changes described

above. Thus, infusions made either dorsal to CeA within substantia innominata or globus pallidus, or lateral to CeA within BLA, did not result in any changes in DA or 5-HT neurotransmission. These results are consistent with recent observations demonstrating that lidocaine injections within CeA attenuate reward-related increases in DA release within NAcc, whereas lidocaine injections within BLA do not attenuate such increases in DA neurotransmission (Ahn and Phillips, 2003). However, the current results are in seeming conflict with previous work demonstrating that electrical stimulation of BLA increases DA release within PFC and NAcc (Floresco *et al*, 1998; Jackson and Moghaddam, 2001; Howland *et al*, 2002). There are several possible reasons for this apparent discrepancy. First, AMPA receptor activation may impact only a subset of BLA neurons. Thus, it is possible that stimulation of AMPA receptors within BLA does not target those neurons which are activated by electrical stimulation and influence dopaminergic neurotransmission. Second, electrical stimulation may activate fibers of passage that drive DA neurotransmission. Finally, electrical stimulation at parameters in the same general range as those used in these previous studies can produce either focal or generalized seizures (Goddard *et al*, 1969; Cain and Corcoran, 1981). These seizures have been elicited with as few as a single episode of stimulation and could increase DA release through a generalized stress response.

In addition to demonstrating a role of CeA in monoaminergic neurotransmission and arousal, the current observations further suggest a critical role of AMPA receptors in these actions. In particular, these observations indicate that CeA-AMPA receptors exert a robust stimulatory action on DA neurotransmission, particularly within PFC, in addition to changing behavioral state. CeA-AMPA receptors have been previously implicated in both the acquisition and expression of fear-potentiated startle (Walker and Davis, 1997, 2002). The current observations suggest the hypothesis that CeA-AMPA receptors contribute to increased rates of DA and 5-HT efflux within PFC and other dopaminergic terminal fields in aversive and/or appetitive environmental conditions.

### Circuitry Underlying CeA-AMPA-Induced Activation of Monoaminergic Systems

The circuitry through which CeA influences DA and 5-HT neurotransmission, as well as CORT secretion, is currently unknown. Previous reports suggest a direct projection from CeA to midbrain dopaminergic nuclei, including both the substantia nigra and the ventral tegmental area (VTA) (Price and Amaral, 1981; Gonzales and Chesselet, 1990; Wallace *et al*, 1992). However, it should be noted that recent studies have failed to find evidence of a direct projection from CeA to VTA (Zahm *et al*, 1999; Fadel *et al*, 2001). Another possibility is that CeA influences dopamine neurotransmission via a multisynaptic pathway to dopaminergic cell bodies. For example, there exists an extensive projection from CeA to the posterior lateral hypothalamus (Gonzales and Chesselet, 1990; Krettek and Price, 1978; Price and Amaral, 1981; Zahm *et al*, 1999; Petrovich *et al*, 2001), which in turn projects to VTA (Phillipson, 1979; Hosoya and Matsushita, 1981; Berk and Finkelstein, 1982). Similarly, CeA could influence serotonergic neuronal

activity via either a direct projection to the dorsal raphe nucleus (Price and Amaral, 1981; Peyron *et al*, 1998) or a multisynaptic pathway, possibly through the lateral hypothalamus (Behzadi *et al*, 1990; Peyron *et al*, 1998; Hosoya and Matsushita, 1981; Berk and Finkelstein, 1982). Finally, a projection from CeA to the paraventricular nucleus of the hypothalamus has been described, through which CeA could influence CORT secretion (Gray *et al*, 1989; Marcilhac and Siaud, 1997; Prewitt and Herman, 1998).

An asymmetrical activation of PFC DA efferents has often been observed in stress (Carlson *et al*, 1993; Sullivan and Gratton, 1998; Berridge *et al*, 1999). For example, novelty-stress elicits a greater increase in DOPAC/DA levels within the right hemisphere PFC than that observed within the left hemisphere (Berridge *et al*, 1999). Currently, the neural basis for asymmetrical DA release within PFC in stress remains unclear. Evidence from the present study suggests that this asymmetrical activation of PFC DA efferents in stress does not involve actions of the CeA. First, bilateral AMPA infusion within CeA elicited comparable activation of right and left PFC DA efferents. Second, unilateral AMPA infusions did not impact indices of DA release within either hemisphere of PFC.

### Conclusions

Recent work suggests a broad modulatory action of the CeA on a variety of cognitive processes under conditions in which information collection is essential (Gallagher and Holland, 1994; Whalen, 1998; Holland *et al*, 2000). Similarly, extensive evidence indicates a critical role of DA (and to a lesser extent 5-HT) in a variety of cognitive and affective processes under both appetitive and aversive high-arousal conditions (Wise and Rompre, 1989; Salamone, 1994; Williams and Goldman-Rakic, 1995; Arnsten, 1997; Schultz, 1998; Lucki, 1998; Smith-Roe and Kelley, 2000; Baldwin *et al*, 2002). The current studies suggest CeA-dependent activation of dopaminergic and serotonergic systems may contribute to optimal behavioral responsiveness and plasticity associated with both appetitive and aversive high-arousal conditions.

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