

# Cortico-accumbens fiber stimulation does not induce dopamine release in the nucleus accumbens in vitro

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**Abstract** Interactions between dopamine (DA) and glutamate in the nucleus accumbens (NA) are important for a variety of cognitive and limbic functions. Although, there is strong evidence that DA controls glutamate responses, the converse (glutamate affecting DA release) is controversial. To determine whether endogenous glutamate released from corticostriatal terminals can evoke DA release by local interactions in the NA, we measured DA release with amperometry simultaneously with whole cell recordings from NA medium spiny neurons (MSNs) in a slice preparation preserving DA terminals (but not cell bodies) and cortico-accumbens fibers. MSNs responded to cortical stimulation with a postsynaptic potential that was blocked by the AMPA antagonist CNQX, but no DA overflow was detected with the carbon fiber electrode. This absence of DA release cannot be accounted for by a deterioration of the DA terminals in this slice preparation since DA release was evoked with a caudal stimulation in the same slices. The DA signal was modulated as expected by bath application of a DA transporter blocker. The data show that cortico-striatal activation does not induce DA release by local interactions, suggesting that observations of glutamate-evoked DA release previously reported in vivo may be taking place via an extra-NA circuit.

**Keywords** Nucleus accumbens · Dopamine · Glutamate · Electrophysiology · Prefrontal cortex · Amperometry

## Introduction

The nucleus accumbens (NA) receives glutamatergic afferents from the prefrontal cortex (PFC) and a dense dopamine (DA) innervation originated in the ventral tegmental area (VTA) (Groenewegen et al. 1999) with terminals in close apposition to glutamatergic afferents (Sesack and Pickel 1990). This arrangement makes the NA suited for a DA modulation of cortical inputs. The outcome of these interactions impacts the output of NA onto other basal forebrain structures, including the ventral pallidum (Heimer et al. 1991), thereby modulating ascending projections to cortical areas (Heimer 2003). Functional interactions between DA and glutamate in the NA are proposed to play a crucial role in reward and goal-directed behaviors (Cheer et al. 2007; Goto and Grace 2005; O'Donnell 2003) and may become dysfunctional in conditions such as drug addiction (Cornish and Kalivas 2000; Kalivas and Volkow 2005; Kelley 2004) and schizophrenia (Grace 2000). Exogenous glutamate and its agonists increase extracellular DA levels in the striatum in vitro (Bowyer et al. 1991; Clow and Jhamandas 1989; Jin 1997; Johnson and Jeng 1991; Marien et al. 1983) and in vivo studies revealed NA DA release in response to PFC stimulation (Taber et al. 1995; Taber and Fibiger 1993). These findings have been interpreted as glutamate locally evoking DA release by a direct action on DA terminals. However, direct evidence of glutamate released from cortical fibers in the NA causing DA release is lacking. Some disease models included the assumption that glutamate released from corticostriatal terminals in striatal regions can induce

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DA release (Grace 1991). Some *in vivo* studies revealed that the PFC-evoked DA release in the NA was dependent on an intact VTA (Taber et al. 1995), suggesting the interaction could not be local. Although the disease models were reformulated to omit local glutamate-induced DA release (O'Donnell and Grace 1998), this concept has remained popular. Indeed, anatomical data argues for PFC fibers synapsing on VTA DA neurons that project back to the PFC, but not to those projecting to the NA (Carr and Sesack 2000), suggesting that the VTA inactivation *in vivo* may have affected NA-projecting DA cells that are not activated by PFC stimulation. In another elegant study, Bamford et al. (2004) failed to observe striatal DA release along with the glutamate released with cortical stimulation, but those experiments were conducted in coronal slices that may have cut the cortical afferents, so the glutamate detected could be originated elsewhere. Here, we explored whether PFC afferents can induce DA release in the NA with intra-NA amperometry recordings simultaneous with whole cell recordings from NA MSN in a slice preparation preserving DA terminals and cortico-accumbens fibers.

## Methods

All experimental procedures were performed according to the USPHS *Guide for Care and Use of Laboratory Animals* and were approved by the University of Maryland School of Medicine Institutional Animal Care and Use Committee. Young adult (50–65 days old) male Sprague Dawley rats (Charles River Laboratories, Wilmington, MA, USA) were maintained on a 12 h light/dark cycle with food and water available *ad lib* until the time of experiment. Rats were deeply anesthetized with chloral hydrate (400 mg/kg, *i.p.*) before decapitation. Brains were removed into ice-cold artificial cerebrospinal fluid (ACSF) containing: 125 mM NaCl, 25 mM NaHCO<sub>3</sub>, 10 mM glucose, 3.5 mM KCl, 1.25 mM NaH<sub>2</sub>PO<sub>4</sub>, 0.5 mM CaCl<sub>2</sub>, 3 mM MgCl<sub>2</sub>; pH = 7.45; osmolarity 295 mOsm. Parasagittal slices (350 μm thick) with a near 10° angle that preserves corticoaccumbens fibers (O'Donnell and Grace 1994) were cut on a Vibratome in ice-cold ACSF. Slices were transferred and incubated in warm (35°C) ACSF solution constantly oxygenated with 95% O<sub>2</sub>–5% CO<sub>2</sub> for at least 1 h before recording. Slices were then transferred to a submersion-type recording chamber maintained at 33–35°C and superfused with oxygenated ACSF at a flow rate of 2 ml/min. In the recording ACSF, CaCl<sub>2</sub> was increased to 2 mM and MgCl<sub>2</sub> was reduced to 1 mM.

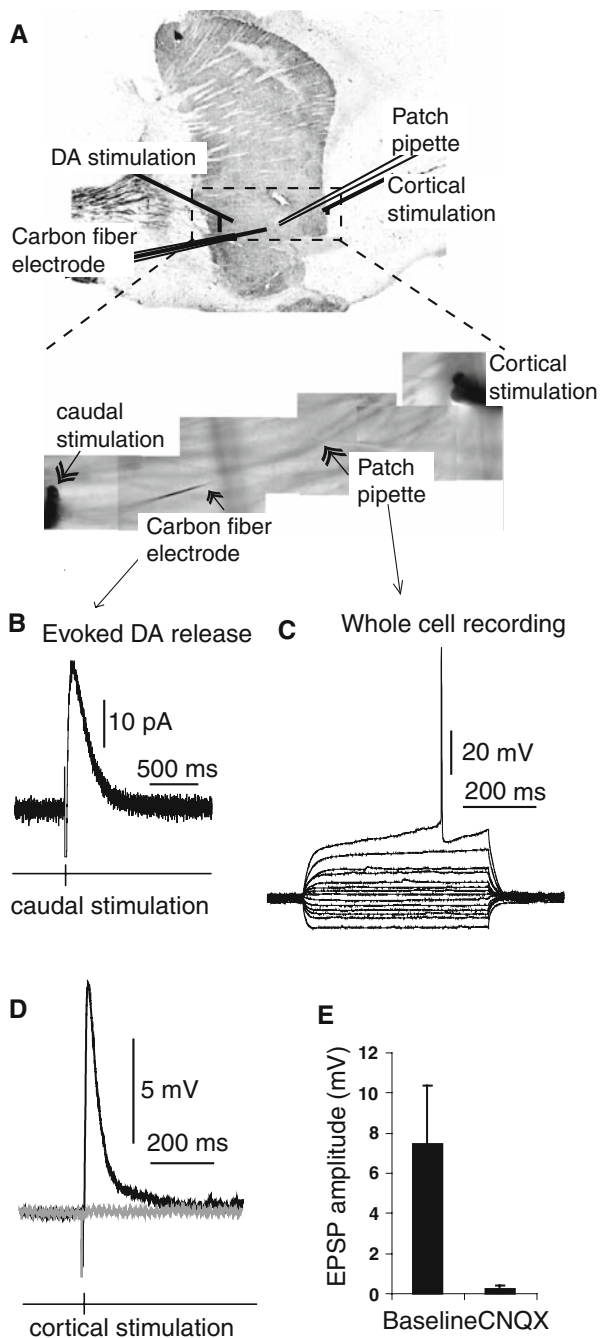
Extracellular DA level variations associated with electrical stimulation of afferent pathways were monitored with a carbon fiber electrode using continuous amperometry at +0.4 V. The active surface of the carbon fiber was 8 μm in

diameter and 100 μm long. DA recordings were acquired with a potentiostat (WPI, Sarasota, FL, USA) connected to an Axoclamp-2A amplifier (Axon instruments, Foster City, CA, USA) and controlled with Axoscope 8.1 (Axon Instruments) at a sampling rate of 10 kHz. A stimulating electrode consisting of a twisted pair of nichrome wires was placed on a location caudal to the recording site at about 1 mm from the recording site. Caudal stimulation was conducted with pulses of 0.5 ms and 0.1–0.3 mA.

Whole-cell current-clamp recordings were performed from MSN within the core region of the NA in the same brain slices. Patch pipettes (5–10 MΩ) were filled with: 115 mM K-gluconate, 10 mM HEPES, 2 mM MgCl<sub>2</sub>, 20 mM KCl, 2 mM Mg-ATP, 2 mM Na<sub>2</sub>-ATP, 0.3 mM GTP (pH 7.3; 280 mOsm). Neurobiotin (0.125%) was added to the internal solution for labeling of the recorded neuron. All drugs were mixed into oxygenated ACSF and applied to the recording solution in known concentrations. Nucleus accumbens MSNs were identified under visual guidance using infrared-differential interference contrast (IR-DIC) video microscopy (Olympus BX50-WI). The image was detected with an IR-sensitive CCD camera (DAGE-MTI) and displayed on a monitor. Whole-cell current-clamp signals were acquired with an Axoclamp-2A amplifier (Axon Instruments), digitized with an A/D converter (Digidata, Axon Instruments) and sampled with Axoscope 8.1 (Axon Instruments) at a rate of 10 kHz. Electrode potentials were adjusted to zero before recording without correcting the liquid junction potential (estimated at 10 mV). Postsynaptic potentials in MSNs were evoked by electrical stimulation of cortico-accumbens fibers in the white matter between the rostral PFC and the NA with 0.2–0.9 mA, 0.5 ms current pulses delivered every 15 s.

## Results

DA overflow and MSN synaptic responses to cortical and caudal intrastriatal stimulation were simultaneously recorded in cortico-accumbens slices obtained from nine adult rats (Fig. 1a). A carbon fiber electrode was used to detect evoked DA release (Fig. 1b) in the vicinity of a patch electrode used for whole-cell recordings from NA MSNs (Fig. 1c). The distance between both electrodes was 50 and 150 μm. Resting membrane potential was  $-76.3 \pm 6$  mV ( $n = 20$ ), and input resistance was  $91.2 \pm 35$  MΩ ( $n = 20$ ). These membrane properties were within the range of what previously reported (Chang and Kitai 1986; Kawaguchi et al. 1989; O'Donnell and Grace 1993, 1994; Pennartz et al. 1992; Uchimura et al. 1989), indicating that MSNs were not affected by the vicinity of the amperometry probe. A stimulating electrode placed caudally (0.75–1.5 mm from the recording site) was used to activate DA



**Fig. 1** Simultaneous recording of evoked DA release and MSN in the NA. **a** TH-stained slice illustrating the position of recording and stimulating electrodes. *Inset* shows IR-DIC image of the slice preparation with the electrodes. Both the patch and the carbon fiber electrodes are located in a line following the cortical fibers entering the NA, and are therefore within the region receiving glutamate released by those afferents. **b** Representative trace of DA overflow evoked by caudal stimulation (1 mA, 0.5 ms). **c** Overlay of whole cell current-clamp responses to depolarizing and hyperpolarizing current pulses (50 pA steps from  $-200$  to  $+300$  pA, 500 ms duration) in a NA MSN. **d** Representative traces of cortical stimulation (0.8 mA, 0.5 ms)—evoked EPSPs in a MSN before (*black trace*) and after CNQX (*gray trace*). **e** Bar graph of EPSP amplitude and its reduction by the AMPA antagonist

fibers, and a stimulating electrode in the forceps minor (1.1–1.5 mm from the recording site) activated cortico-accumbens fibers. Cortical stimulation evoked excitatory postsynaptic potentials (EPSPs) in MSN with amplitudes ranging from 4.8 to 18.6 mV (Fig. 1d). Caudal stimulation also evoked EPSPs ranging from 4.3 to 11.4 mV. Bath application of the AMPA antagonist CNQX (5  $\mu$ M) nearly abolished EPSPs evoked by the cortical stimulation (Fig. 1d, e) as well as those evoked by caudal stimulation (not shown), indicating that cortico-accumbens and caudal afferent fibers provide glutamate innervation in a parasagittal slice preparation.

Evoked DA release was recorded by continuous amperometry with a carbon fiber electrode positioned in the NA while stimulating cortical or caudal afferents in the slice. Caudal stimulation evoked transients in the carbon fiber ranging from 12.8 to 64 pA. To verify those transients were generated by DA and not other reactive species, the DA transporter (DAT) blocker nomifensine (5  $\mu$ M) was applied to the bath and stimulation protocols repeated. Nomifensine increased half-life of DA overflow from  $64 \pm 23$  to  $1,087 \pm 377$  ms ( $P = 0.003$ ,  $n = 5$ ; Fig. 2a, b) and its peak amplitude from  $39.2 \pm 13.1$  to  $71.4 \pm 21.4$  pA ( $P = 0.002$ ,  $n = 5$ ; Fig. 2a, c). These data suggest that the transients detected following stimulation were indeed DA.

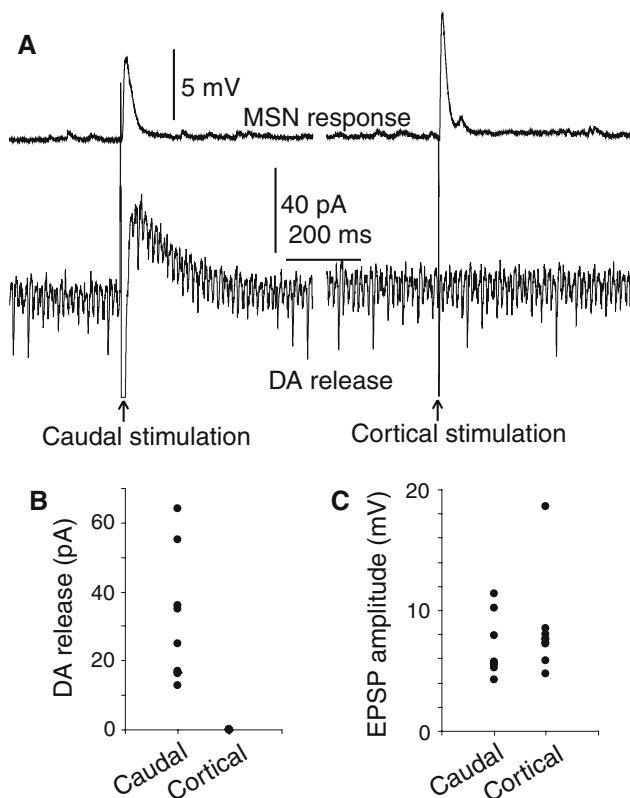
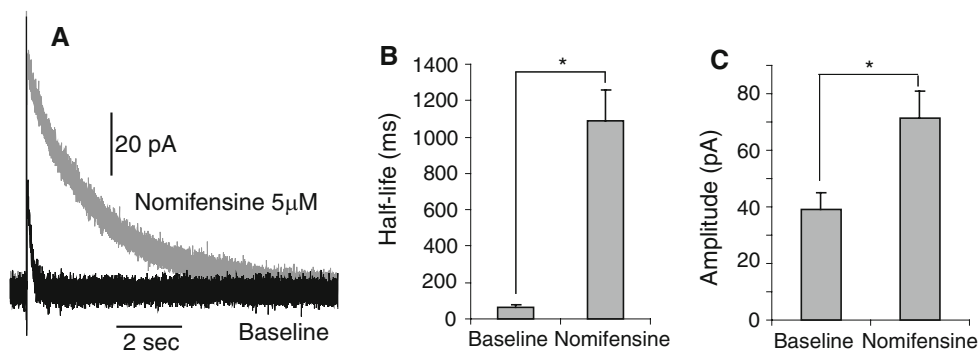
Cortical stimulation, on the other hand, consistently failed to evoke DA overflow, even when caudal intraatrial stimulation induced DA release in the same slice (Fig. 3a, b). In all cases, both cortico-accumbens and caudal stimulation evoked EPSPs, but a DA transient was only detected with caudal stimulation (Fig. 3a–c). Thus, despite effective local DA release capability, endogenous glutamate release from cortico-accumbens fibers did not evoke DA release.

## Discussion

We conducted whole-cell recordings from NA MSN simultaneously with detection of DA levels with continuous amperometry in cortico-accumbens slices obtained from adult rats. Intra-accumbens stimulation at a site caudal to the recording area elicited AMPA-dependent EPSPs and DA overflow in the NA. Electrical stimulation of cortical afferents evoked an AMPA postsynaptic potential in the MSN, but did not induce DA overflow.

Caudal stimulation evoked AMPA EPSPs with amplitudes within the range of those recorded by PFC stimulation. Several excitatory fibers may be traversing the area stimulated towards the NA. These include afferents from the BLA, PPN and a recently reported subset of

**Fig. 2** Modulation of DA overflow by nomifensine. **a** Overlay of DA transients before and after Nomifensine bath application. **b** Bar graph showing the effect of nomifensine on half-life of DA overflow (ms). **c** Bar graph showing the effect of nomifensine on DA overflow amplitude (pA). (\* $P < 0.003$ )



**Fig. 3** Simultaneous DA release and EPSPs evoked by cortical and caudal stimulation. **a** Representative traces of whole-cell recordings (top) and DA amperometric signal (bottom) evoked by caudal stimulation (left) and cortical stimulation (right). **b** DA overflow amplitude in response to caudal or cortical stimulation for all slices tested ( $n = 9$ ). **c** Amplitude of EPSPs evoked with caudal or cortical stimulation ( $n = 9$ )

projection neurons in the VTA that release glutamate (Yamaguchi et al. 2007). The possibility that these EPSPs are due to antidromic activation of corticofugal fibers that leave collaterals in the NA cannot be ruled out, although it is unlikely that such projections and their collaterals would remain in the plane of slicing as often as suggested by the frequency of responses. Thus, caudal stimulation evoked a glutamatergic response of unknown origin as well as a reliable DA transient.

Cortical stimulation, on the other hand, failed to evoke a DA signal. Such absence of DA response to cortical stimulation could be due to absence or ineffectiveness of DA or cortical terminals in our preparation. These possibilities are unlikely, however, as we were able to elicit DA release by stimulating the caudal aspect of the slice in the same recording sessions in which a cortical stimulation that effectively evoked EPSPs failed to evoke a DA response. Moreover, the recorded DA signal was modulated as expected by DAT blockade. These results indicate that DA terminals are present in our slice preparation and can release DA when stimulated. An inefficient cortico-NA transmission can also be ruled out. Recordings from MSNs in the vicinity of the carbon fiber electrode revealed EPSPs that were blocked by CNQX following both cortical and caudal stimulation. This observation shows that cortical stimulation elicited glutamate release in the vicinity of the region in which DA release was detected with caudal stimulation. It remains to be determined whether cortical activity with bursts of action potential, as observed in the PFC of awake animals (Peters et al. 2005), could impact DA terminals differently and cause some extent of local DA release.

All evoked responses in this study involve activation of long-distance projections and neurotransmitter release. Unlike most previous studies where stimulating electrodes were placed in the vicinity of carbon fibers or patch recordings (Jones et al. 1998; Schmitz et al. 2002), our experiments were conducted with a near-1 mm separation between stimulation and recording sites. This ensures that evoked responses are due to action potentials traveling down projection fibers and activating physiological release mechanisms, and prevents the potential confound of current invading the terminals causing transmitter release independent of action potential arrival and therefore not likely to be modulated by physiological mechanisms.

Overall, the data suggest that previous findings of DA release by cortical activation may have been due to activation of poly synaptic circuits. This is consistent with observations that inactivation of the VTA abolished cortical stimulation-induced DA release in the NA (Taber et al.

1995), and in vitro findings reporting that potassium evoked glutamate does not induce DA release (Bowyer et al. 1992). Thus, PFC activity could affect subcortical DA, but it is not likely due to a direct impact on NA dopaminergic terminals. Tonic DA release, therefore, may depend on a pacemaker style firing of VTA neurons rather than glutamate in the vicinity of the terminals. Limbic afferent stimulation, on the other hand, has been shown to elicit local DA release independently of the VTA (Floresco et al. 1998). This suggests that emotional/contextual (limbic) input to the NA could drive DA release directly in the NA, but the impact of the medial PFC is more likely dependent on modulation of DA cell activity.

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