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BRAIN STIMULATION REWARD IS ALTERED BY AFFECTING DOPAMINE–GLUTAMATE INTERACTIONS IN THE CENTRAL EXTENDED AMYGDALA

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Abstract—This work compares the effects on brain stimulation reward (BSR) when combining D2 dopamine receptor and AMPA glutamate receptor manipulations in the sublenticular central extended amygdala (SLEAc) and the nucleus accumbens shell (NAc shell). Thirty-seven male Long Evans rats received medial forebrain bundle (MFB) stimulation electrodes and bilateral injection guide cannulae aimed at either the SLEAc or the NAc shell. The rate-frequency paradigm was used to assess drug-induced changes in stimulation reward effectiveness and in response rate following 0.5 µl infusions of 0.50 µg of 1,2,3,4-tetrahydro-6-nitro-2, 3-dioxo-benzo[*f*]quinoxaline-7-sulfonamide (NBQX) (AMPA receptor antagonist), 10.0 µg of quinpirole (D2 receptor agonist), 0.25 µg of AMPA (AMPA receptor agonist), 3.0 µg of eticlopride (D2 receptor antagonist), 0.50 µg of NBQX with 10.0 µg of quinpirole, and 0.25 µg of AMPA with 3.0 µg of eticlopride. The drugs were injected both ipsi- and contralateral to the stimulation site. AMPA blockade and D2 stimulation synergized to reduce BSR's reward efficacy when directed at the SLEAc contralateral to the stimulation site whereas changes in reward efficacy were primarily D2-dependent following injections into the ipsilateral SLEAc. When injected into the NAc shell the drugs had only one significant effect on the frequency required to maintain half-maximal responding: injections of NBQX with quinpirole ipsilateral to the stimulation site increased required frequency significantly more than did injections of saline. Contrary to expectations, stimulating AMPA receptors with and without co-blockade of D2 receptors also decreased the stimulation's reward efficacy, although these effects may reflect general behavioral disruption more than effects on reward per se. These results indicate a role for the SLEAc in BSR and also suggest that SLEAc neurons ipsi- and contralateral to the stimulated MFB play their roles in BSR through different mechanisms. © **2012 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: extended amygdala, nucleus accumbens, reward, dopamine, glutamate.

INTRODUCTION

Our lab has presented evidence that the central sublenticular extended amygdala (SLEAc) is involved in the rewarding effect of medial forebrain bundle (MFB) stimulation (Waraczynski, 2003, 2008) and may be more important to that phenomenon than is the shell of the nucleus accumbens (NAc) (Waraczynski et al., 2010). The SLEAc has extensive projections via the MFB to multiple structures known to support self-stimulation whereas the NAc shell's projections are comparatively limited. Mapping and metabolic marker studies show that the SLEAc and its interconnections support and are activated by rewarding MFB stimulation and its projection axons have conduction capabilities consistent with the axons known to be activated by rewarding MFB stimulation (see Waraczynski (2006) for a thorough review of these data). This report further supports our conjecture that the SLEAc is an important component of the substrate for brain stimulation reward (BSR).

In a recent study, stimulation or blockade of dopaminergic D2 receptors in the SLEAc modestly reduced or slightly augmented, respectively, stimulation reward efficacy. No such effects were found following D2 stimulation or blockade in the NAc shell or from affecting D1 receptors in either structure (Waraczynski et al., 2010). The modest nature of the SLEAc-based effects suggests that dopaminergic influences might have to combine with some other process to substantially alter the activity of reward-relevant SLEAc neurons. Glutamatergic neurotransmission is a strong candidate for such a process.

Although there has been little study of the medium spiny neurons (MSNs) populous in the SLEAc, there is a rich literature on the interaction between dopaminergic and glutamatergic communication in striatal MSN's (see Jones (2010) for recent reviews). Because our previous work found stronger effects from manipulating D2 vs. D1 receptors they will be our focus here. In the striatum, D2 receptors interact with glutamate-mediated excitation in several ways. Postsynaptically, D2 stimulation reduces protein kinase A (PKA) levels via reduction of cyclic adenosine monophosphate (cAMP). Loss of PKA reduces the phosphorylation of AMPA glutamate receptors at the serine 845 residue and thus impairs excitatory postsynaptic currents through those receptors

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Abbreviations: BSR, brain stimulation reward; cAMP, cyclic adenosine monophosphate; MFB, medial forebrain bundle; MSN, medium spiny neuron; NAc, nucleus accumbens; NBQX, 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[*f*]quinoxaline-7-sulfonamide; PKA, protein kinase A; SLEAc, central sublenticular extended amygdala.

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(Roche et al., 1996; Hernandez-Echeagaray et al., 2004). D2 stimulation also reduces MSN excitability by enhancing outward rectifying K^+ currents that counter glutamate's excitatory effects (Nisenbaum and Wilson, 1995; Wilson and Kawaguchi, 1996) and by suppressing excitatory Ca^{2+} currents through L-type Ca^{2+} channels (Hernandez-Lopez et al., 2000). Presynaptically, stimulating D2 receptors reduces glutamate release (Lin et al., 2003; Bamford et al., 2004), possibly through retrograde signaling via endocannabinoids rather than direct action at D2 receptors on glutamatergic terminals (Yin and Lovinger, 2006). Stimulating D2 receptors should therefore render MSNs less excitable and/or active, and should thus decrease MFB stimulation's reward efficacy. Blocking D2 receptors should have the opposite effect. Also, blocking AMPA receptors should render MSNs less active and MFB stimulation less rewarding, while stimulating those receptors should have the opposite effect.

This work focuses on the interaction between D2 receptors and AMPA receptors, as opposed to other glutamate receptors, because of AMPA receptor involvement in fast postsynaptic depolarization. While dopamine also modulates postsynaptic responses to NMDA receptor stimulation, NMDA receptors do not seem to be heavily implicated in fast postsynaptic depolarization (Cherubini et al., 1988; Nisenbaum et al., 1993). NMDA receptors have to be released from Mg^{2+} block via membrane depolarization before they can contribute to postsynaptic responses. The Ca^{2+} currents they mediate may be more involved in synaptic plasticity than in transient alterations of membrane voltage (for a recent review of NMDA receptors' role in synaptic plasticity in striatal MSNs, see Klug et al., 2010). Furthermore, D2 receptors seem to have a greater influence on postsynaptic response to AMPA receptor stimulation than to NMDA stimulation, while NMDA receptors seem more influenced by D1 receptors (Cepeda et al., 1993; Levine et al., 1996; see also Moyer et al., 2007).

Given our working hypothesis that D2-mediated mechanisms must combine with glutamatergic influences before MSN activity is notably affected, and our concurrent hypothesis that reward function depends more on the activity state of MSNs in the SLEAc than in the NAc shell (Waraczynski et al., 2010), we make the following predictions: (1) blocking AMPA receptors and stimulating D2 receptors should reduce BSR, particularly when they are done simultaneously; (2) stimulating AMPA receptors and blocking D2 receptors should have the opposite effect; (3) these effects will be more pronounced when drugs are injected into the SLEAc vs. the NAc shell.

EXPERIMENTAL PROCEDURES

Subjects and surgery

Thirty-seven male Long-Evans rats, weighing approximately 350–400 g at the time of surgery, received unilateral rostral and caudal MFB stimulation electrodes and bilateral guide cannulae aimed at either the SLEAc or the NAc shell. The coordinates for the rostral stimulation electrodes were 2.8 mm caudal to bregma, 1.7 mm from the midline, and 7.7 mm ventral to the dura. These electrodes were aimed at the MFB as it courses through the lateral hypothalamus. The coordinates for the caudal electrodes were 4.5 mm caudal to bregma, 0.9 mm from the midline, and 7.8 mm ventral to the dura. These electrodes were aimed at the MFB as it courses through the ventral tegmental area. Both electrodes were implanted in the same hemisphere. The coordinates for the SLEAc cannulae were 1.3 mm caudal to bregma, 2.5 mm lateral to the midline, and 6.3 mm ventral to the dura. The coordinates for the NAc cannulae were 1.5 mm caudal to bregma, 0.80–1.0 mm lateral to the midline, and 5.5–5.8 mm ventral to the dura. Twenty-four rats received SLEAc cannulae and 13 rats received NAc cannulae. More cannulae were targeted at the SLEAc because that structure is considerably smaller than the NAc and therefore it was more likely that some of those cannulae might miss their target.

The reason for implanting two stimulation electrodes was purely pragmatic. Implanting two electrodes increased the chances that a rat would self-stimulate, with one electrode serving as a backup should the other be off target. The electrodes were made from 0.25-mm diameter stainless steel wire insulated except at the tip, which was sanded to a circular cross section. A machine screw in the skull served as the stimulation anode. The cannulae were made from 23-gauge stainless steel tubing 12 mm long and were implanted such that the tip was placed 1 mm above the injection target. The cannulae were blocked with stylets made from 30-gauge tubing.

The rats were housed individually with food and water continuously available in a day/night reversed colony. All testing was conducted during the dark phase of the cycle. The rats were kept in accordance with institutional and governmental animal care and use guidelines.

Procedures

Rate-frequency testing. During rate-frequency testing the rat was placed in an operant chamber with a lever protruding from one wall. The rat was connected to a Stimtek ST1200 stimulation generator (San Diego Instruments) via a flexible cable and commutator (Plastics One). All experimental events were controlled by Stimtek ST1000 CPU and ST1100 I/O boards in communication with a master PC.

After three to five days postsurgical recovery the rats were trained to press a lever for a 0.5 s train of 0.1 ms cathodal pulses delivered by a constant current generator. The rats were trained using stimulation of either the rostral or caudal MFB site, whichever supported the more robust responding with minimal motoric or aversive collateral effects. Once the lever press response was reliably established (i.e., the rats would press without coaching for stimulation delivered on a variable interval 3-s schedule) rate-frequency testing began.

Each point in a single rate-frequency curve was determined as follows: For 50 s, the rat was allowed to press for 0.5-s trains of pulses of a given frequency, delivered on a variable interval 3-s schedule. At the start of each 50-s trial the rat received three non-contingent trains of the stimulation that would be available during that trial. Data from the first 10 s of the trial were discarded to allow response rate to adjust to the available frequency; response rate over the last 40 s was recorded.

The rats were tested at three stimulation current intensities – 200, 400, and 800 μA – to maximize the chances of detecting any drug effects. In some rats the infusion may affect neural elements that are associated with axons lying close to the stimulation electrode tip. In these rats, effects should be detected at lower currents, but may be lost as a larger population of reward-relevant neurons is recruited at higher currents. In other rats the infusion may affect neural elements associated with axons lying distal to the electrode tip. In these rats we may not detect effects at low currents but see them at higher currents. Three curves were collected at each current on each test day.