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Lesion of the ventromedial nucleus of the thalamus blocks acute cocaine-induced changes in striatal glutamate

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Recent reports characterizing the thalamic inputs to the striatum have increased interest in thalamostriatal circuitry (Raju et al., 2006; Lacey et al., 2005). Traditionally, the primary source of glutamate in the dorsolateral striatum (DLS) has been attributed to projections originating from the sensorimotor cortex (McGeorge and Faull, 1989). These corticostriatal terminals form excitatory synaptic contacts onto the spines of medium spiny neurons (MSNs) (Smith and Bolam, 1990), which project to the substantia nigra pars reticulata (SNr) and entopeduncular (EPN) nucleus. The SNr and EPN have GABAergic projections to the ventromedial (VM) nucleus of the thalamus (Kha et al., 2000), which is considered the primary motor thalamic nucleus in the rat (Groenewegen et al., 1999). The VM sends a glutamatergic projection to the sensorimotor cortex, which affects the cortical input to the DLS.

Attention has also focused on thalamic inputs to the striatum arising from the intralaminar parafascicular nuclei, the relay thalamic nuclei, and midline thalamic nuclei (Lacey et al., 2005; Smith et al., 2004). Specifically, thalamic relay nuclei such as the VM form primarily axospinous contacts onto striatal neurons (Raju et al., 2006). These thalamostriatal neurons are identified by vesicular glutamate transporter 2 immunolabeling and are likely glutamatergic (Lacey et al., 2005; Raju et al., 2006).

Striatal glutamate is profoundly affected by changes in the VM. A VM lesion decreases striatal glutamate (Touchon et al., 2004) and GABA injections into the VM increased extracellular glutamate in the striatum (Meshul et al., 1996). We have reported that extracellular glutamate within the rat DLS increases significantly as compared to controls 1 day after a single injection of cocaine (McKee and Meshul, 2005). Therefore, we hypothesized that this cocaine-induced increase in striatal glutamate may result from activation of the VM via the thalamo-corticostriatal or thalamostriatal pathways. By lesioning the VM, the indirect thalamocorticostriatal and the direct thalamostriatal projection would be eliminated enabling us to investigate the role of the VM in the cocaine-induced

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increase in striatal glutamate. To do this, we measured changes in extracellular glutamate in both VM and sham-lesioned animals 1 day after cocaine treatment.

Subjects were male Sprague-Dawley rats (3 months old, Harlan, Indianapolis, IN). Animals were maintained in a temperature and light controlled room on a 12-hour light/ dark cycle, with access to food and water *ad libitum*. All procedures complied with the *NIH Guide for Care and Use of Laboratory Animals* and were approved by the Veteran's Affairs Medical Center's IACUC.

All animals subjected to electrolytic lesions simultaneously underwent guide cannula implantation to reduce the number of surgeries. Rats were anesthetized and their shaved heads secured via placement in a Stoelting stereotaxic apparatus (McKee and Meshul, 2005). Two holes were drilled through the exposed skull above the VM for electrode placement. The following coordinates were used to effectively lesion the rostral-caudal extent of the VM: -2.3 and -2.7 mm rostral, ± 1.4 mm lateral, and -7.4 mm ventral with respect to bregma (Paxinos and Watson, 2005). A SNE-300 stainless steel monopolar electrode with a 0.1 mm tip was lowered into the VM nucleus (Rhodes Medical Instruments, Woodland Hills, CA) and 0.5 mA current was applied for 2 seconds (UGO Basile). Sham lesioned animals had the hole drilled into the skull but the needle was not inserted into the brain.

Immediately after the electrolytic lesion surgery was completed, a hole was drilled at the following coordinates: (mm from Bregma) $+0.5$ rostral and $+3.1$ lateral (Paxinos and Watson, 2005). A stainless steel guide cannulae (15 mm long, 20-gauge) (Small Parts, Miami Lakes, FL) was lowered 0.5 mm without penetrating the dura as detailed previously (McKee and Meshul, 2005). The cellulose tips of dialysis probes were 2 mm in length to effectively target only the dorsolateral quadrant of the striatum. In the morning before microdialysis, rats were injected with 15 mg/kg cocaine hydrochloride i.p. (Coc) (Sigma-Aldrich, St. Louis, MO) dissolved in sterile water or 0.2 ml sterile water for the vehicle-treated (Veh) animals. A detailed description of the microdialysis methods have been reported in McKee and Meshul (2005). Four, 15-minute baseline samples were collected, analyzed for the concentration of glutamate within the dialysate samples using HPLC fluorescence detection, and averaged across groups for each baseline sample time point. Glutamate levels were analyzed using a one-way ANOVA followed by Tukey-Kramer post-hoc test to compare differences between groups.

After the completion of microdialysis, rats were transcardially perfused with fixative as previously described (McKee and Meshul, 2005). The rostral striata and VM were cut into 100 and 60 μ m thick sections, respectively, using a vibratome and stained with thionin to histologically verify the probe placement and lesion damage, respectively. Placements not within the DLS were excluded from the study. The total area of the bilateral VM nuclei lesion was determined to be 61% using the Imago-Pro software (Media Cybernetics, Silver Springs, MD, Version 3.01). The extent of the lesion was calculated for the left and right VM nuclei as the percent of the lesioned area divided by the total area of the VM.

A representative example of the bilateral VM lesion is demonstrated in Figure 1. Figure 2 shows the extent of the electrolytic lesion for all animals. The electrical current produced a significant lesion of the VM such that the area surrounding the site of the lesion showed gliotic changes. Neurons in the most posterior aspect of the VM and the mammillothalamic tract was often lesioned because of its medial location to the VM. There were no obvious behavioral changes between VM-lesioned and sham-lesioned rats.

The probe placements for the rats used in the microdialysis experiment are illustrated in Figure 3. Figure 4 shows the effects of a VM lesion on extracellular glutamate in the rat

DLS 1 day after acute cocaine administration. A one-way ANOVA analysis revealed significant differences between groups [$F(3,12) = 88.3, p < 0.0001$]. Posthoc analysis revealed significant differences between the Sham/Veh and Sham/Coc groups, the VM/Veh and VM/Coc groups, as well as between the Sham/Veh and both lesioned groups.

The VM lesion significantly reduced extracellular glutamate by 39% in the DLS in the cocaine naïve animals (Sham/Veh vs. VM/Veh). Extracellular glutamate was significantly increased by 29% one day after a single injection of cocaine in the sham-lesioned rats, which is consistent with our previous report (Sham/Coc vs. Sham/Veh) (McKee and Meshul, 2005). However, striatal glutamate was significantly decreased in VM-lesioned rats 1 day after a single cocaine exposure as compared to sham-lesioned rats by 46% (VM/Coc vs. Sham/Coc). Hence, the VM lesion blocked the cocaine-induced increase in striatal glutamate. The baseline values in the control groups that received a vehicle injection 1 day prior to microdialysis are consistent between this study and our previous report (McKee and Meshul, 2005).

None of the areas surrounding the VM have connections to the DLS or to the sensorimotor cortex except for the ventrolateral nucleus (VL) of the thalamus, which also projects to the sensorimotor cortex and was considered an acceptable target. A previous report showed an increase in extracellular glutamate in the DLS after GABA was injected into the VM nucleus, which concurs with our lesion results (Meshul et al., 1996). In contrast, when GABA was injected into the adjacent somatosensory (VPM/VPL) nuclei of the thalamus, there was no change in striatal glutamate. Therefore, any damage to the areas surrounding the VM nucleus should not have altered the results.

A VM lesion blocks elevated striatal glutamate 1 day after a single cocaine treatment and restores striatal glutamate levels back to the level of vehicle-treated rats, suggesting that cocaine increases striatal glutamate via a trans-synaptic mechanism in the basal ganglia circuitry. This study highlights the importance of the VM in mediating the effects of cocaine, and further suggests an important role for the thalamocortico-striatal and thalamostriatal pathways. Cocaine blocks dopamine transporters, producing a rise in elevated striatal dopamine, and activates dopamine D1 receptors on MSNs. Activated MSNs inhibit the SNr and EPN, and in turn disinhibit the VM. This could result in an increase in thalamocortico-striatal or thalamocortical activity, which effectively increases extracellular glutamate in the DLS. Circuit-based changes resulting from a single cocaine injection may be particularly relevant given that extracellular glutamate in the DLS may mediate drug-related behaviors like stereotypy (Karler and Calder, 1992; Schmidt, 1986).

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Figure 1.

A representative photomicrograph of a thionin-stained cross section shows the bilateral VM lesion. The white arrow points to the needle tract and a white circle is drawn around the right VM nucleus. The mammillothalamic tract (mt) is labeled for orientation.

Figure 2. Schematic representations illustrate the extent of the electrolytically lesioned VM nucleus of the thalamus. The black area represents cell loss and the gray area shows gliotic changes. The drawings are presented with permission from Paxinos and Watson, 2005, with posted coordinates mm from bregma.

Figure 3.

A schematic representation shows the locations of the microdialysis probe placements for all rats and are presented with permission from Paxinos and Watson, 2005. Probe locations ranged from 1.2 to 0.2 mm anterior to bregma.

Figure 4.

Baseline levels of extracellular glutamate 1 day after a single i.p. injection of 15 mg/kg cocaine. The VM lesion reduced extracellular glutamate in vehicle-treated rats as compared to sham-lesioned, vehicle-treated rats [VM/Veh (n=9) vs. Sham/Veh (n=10), **]. Cocaine significantly increased extracellular glutamate in sham-lesioned rats [Sham/Coc (n=10) vs. Sham/Veh (n=10), *]. However, the VM lesion prevented the cocaine-induced increase in extracellular glutamate [VM/Coc, (n=8) vs. Sham/Coc (n=10), ***]. Each bar represents extracellular glutamate in pmol/μl $\pm$ S.E.M.