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Dopamine dynamics and cocaine sensitivity differ between striosome and matrix compartments of the striatum

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ABSTRACT

The striatum is typically classified according to its major output pathways, which consist of dopamine D1 and D2 receptor-expressing neurons. The striatum is also divided into striosome and matrix compartments, based on the differential expression of a number of proteins, including the mu opioid receptor, dopamine transporter (DAT), and Nr4a1 (nuclear receptor subfamily 4, group A, member 1). Numerous functional differences between the striosome and matrix compartments are implicated in dopamine-related neurological disorders including Parkinson's disease and addiction. Using Nr4a1-eGFP mice, we provide evidence that electrically evoked dopamine release differs between the striosome and matrix compartments in a regionally-distinct manner. We further demonstrate that this difference is not due to differences in inhibition of dopamine release by dopamine autoreceptors or nicotinic acetylcholine receptors. Furthermore, cocaine enhanced extracellular dopamine in striosomes to a greater degree than in the matrix and concomitantly inhibited dopamine uptake in the matrix to a greater degree than in striosomes. Importantly, these compartment differences in cocaine sensitivity were limited to the dorsal striatum. These findings demonstrate a level of exquisite microanatomical regulation of dopamine by the DAT in striosomes relative to the matrix.

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1. Introduction

The striatum has been characterized according to its two major output pathways: the direct pathway (dopamine D1 receptor-expressing neurons) and the indirect pathway (dopamine D2 receptor-expressing neurons) (Gerfen et al., 1990). The striatum can also be classified into two neurochemically-distinct compartments, the striosome and matrix, based on the differential expression of several proteins, including the mu opioid receptor (MOR), acetylcholinesterase, dopamine transporter (DAT), and Nr4a1 (nuclear receptor subfamily 4, group A, member 1) (Crittenden and Graybiel, 2011; Davis and Puhl, 2011; Graybiel and Ragsdale, 1978; Herkenham and Pert, 1981). The matrix compartment comprises approximately 85% of the striatum (Johnston et al., 1990; Mikula et al., 2009) and functions as part of the sensorimotor and associative circuits; in contrast, the striosome compartment comprises approximately 15% of the striatum and is associated with limbic circuits (Eblen and Graybiel, 1995; Gerfen, 1984; Jimenez-Castellanos and Graybiel, 1987; Kincaid and Wilson, 1996). However, these ratios vary across the rostro-caudal extent of the striatum (Davis and Puhl, 2011). Striosome projection neurons are primarily direct pathway neurons developmentally, while matrix projection neurons are either direct or indirect pathway (Fujiyama et al., 2011), though some controversy remains and may be attributable to species differences (Levesque and Parent, 2005). Interestingly, it is theorized that only striatal projection neurons originating in striosomes innervate the substantia nigra pars compacta (SNc) and in this way may globally influence dopamine release in the striatum (Fujiyama et al., 2011; Gerfen et al., 1987; Watabe-Uchida et al., 2012).

Midbrain dopaminergic innervation of the striatum follows a topographical pattern where the dorsal striatum (DS) is innervated primarily by the substantia nigra (and to a lesser extent the lateral ventral tegmental area) and the ventral striatum (VS) is innervated primarily by the ventral tegmental area (VTA) (Beier et al., 2015; Ikemoto, 2007; Lammel et al., 2008). The dopaminergic innervation of the matrix compartment reflects this topographical pattern,

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but striosomal dopamine originates largely from the SNc and to a lesser extent the substantia nigra pars reticulata (Gerfen et al., 1987; Jimenez-Castellanos and Graybiel, 1987; Langer and Graybiel, 1989; Prensa and Parent, 2001). Related to these differences in compartmental dopamine innervation, numerous animal models of Parkinson's disease and dystonia show preferential loss of dopamine terminals or striatal projection neurons in either the striosome or matrix compartments (see Crittenden and Graybiel (2011) for review). Similarly, imbalances in chronic drug-induced immediate early gene expression between striosome and matrix compartments underlie psychostimulant-induced stereotypies (Canales and Graybiel, 2000; Capper-Loup et al., 2002; Jedynak et al., 2012). Given the differences in compartmental dopamine origin and the putative roles of striosome and matrix compartments in numerous dopamine-related neurological disorders, we hypothesized that dopamine signaling between striatal compartments would differ.

Nr4a1 (also known as Nur77 and NGFIB; Entrez Gene ID: 15370) is a nuclear receptor protein initially identified in silico by Davis and Puhl (2011) as a marker for striosomes. Therefore, in the current study, we used fast-scan cyclic voltammetry (FSCV) and Nr4a1-eGFP transgenic mice to identify striosomes and to directly compare dopamine release between the striosome and matrix compartments of the striatum. We found that electrically evoked dopamine release differed between striosome and matrix compartments in a region-specific manner and that these differences could not be attributed to nicotinic acetylcholine receptor (nAChR) antagonism or dopamine D2 autoreceptor inhibition of the dopamine terminal. We further found that cocaine-enhanced dopamine levels and uptake inhibition differed between striosome and matrix compartments in the dorsal, but not ventral, striatum. These findings demonstrate a previously undescribed compartment difference in cocaine mediated regulation of dopamine dynamics in the striatum.

2. Methods

2.1. Subjects

Nr4a1-eGFP mice were obtained from GENSAT and backcrossed with C57BL6J mice (The Jackson Laboratory) for at least six generations. At weaning, visual genotyping with a blue LED and GFP filter-equipped goggles (Electron Microscopy Sciences) was performed. $Drd2^{loxp/loxp}$ mice were obtained from The Jackson Laboratory and crossed with Adora2A-Cre mice (KG139, GENSAT). The $A2ACre/Drd2^{loxp}$ pups were then crossed with $Drd2^{loxp/loxp}$ mice to generate $A2ACre/Drd2^{loxp/loxp}$ (KO) and $Drd2^{loxp/loxp}$ mice (WT). Two to five month old mice of both sexes were used for all experiments. All experimental procedures were approved by the National Institute on Alcohol Abuse and Alcoholism Animal Care and Use Committee and were performed in accordance with National Institutes of Health guidelines.

2.2. Immunofluorescence

Mice were anesthetized with pentobarbital and transcardially perfused with a 4% formaldehyde/PBS solution. The brains were then extracted and post-fixed overnight before being stored in PBS for later sectioning. Forty micron thick sections were blocked in a 5% bovine serum albumin, 0.2% Triton X-100, PBS solution for 4 h at room temperature. The sections were then incubated in a primary antibody/PBS solution overnight as follows: chicken anti-GFP (1/2000; Abcam, ab13970), rabbit anti-MOR (1/4000; Immunostar, 24216), rabbit anti-D2DR (1/500; Frontier Institute, D2R-Rb-Af750), and rat anti-DAT (1/5000; Millipore, MAB369). Sections were then washed three times in 0.2% Triton X-100/PBS before being incubated in secondary antibody solutions with Alexa488 goat anti-chicken (1/2000), Alexa568 goat anti-rabbit (1/1000), or Alexa568 goat anti-rat (1/1000) overnight (secondary antibodies were from Life Technologies). Following five washes in PBS, sections were mounted onto subbed slides, coverslipped with Fluoromount G (Electron Microscopy Sciences), and imaged with a Zeiss Lumar stereoscope and an Axiovert 200 microscope equipped with DAPI, eGFP, and Cy3 filter sets and an Axiocam MR fluorescence camera with Axiovision software (Zeiss).

2.3. Ex vivo slice preparation and fast-scan cyclic voltammetry

Mice were anesthetized with isoflurane and rapidly decapitated. Brains were extracted and immersed in ice-cold, carbogen-saturated (95% O_2/5% CO_2), cutting ACSF containing the following (in mM): Sucrose (194), NaCl (30), KCl (4.5), $NaHCO_3$ (26), NaH_2PO_4 (1.2), dextrose (10), and $MgCl_2$ (1). Coronal sections (300 μm) spanning the striatum were prepared as previously described (John and Jones, 2007) and incubated for 1 h before experiments in carbogen-saturated voltammetry recording ACSF (pH 7.4) containing (in mM): NaCl (126), KCl (2.5), $NaHCO_3$ (25), NaH_2PO_4 (1.2), dextrose (10), HEPES (20), $CaCl_2$ (2.4), $MgCl_2$ (1.2), and L-ascorbic acid (0.4). Nr4a1-eGFP fluorescence was observed using a Stereo-Discovery V8 microscope with a GFP filter set (Zeiss) and an X-cite 120 fluorescence illuminator (Lumen Dynamics). One millisecond, monophasic electrical pulses were generated with a DS3 Constant Current Stimulator (Digitimer) and delivered through a twisted, bipolar, stainless steel stimulating electrode (Plastics One) placed 300 μm from the intended recording sites as shown in Fig. 2A. The distance between the two poles of the stimulating electrode was adjusted to 250 μm.

Carbon fiber electrodes were made as previously described (Crowley et al., 2014) and cut to 80–120 μm. The carbon fiber electrode potential was linearly scanned as a triangle waveform from −0.4 to 1.2 and back to −0.4 V at 400 V/s. Cyclic voltammograms were collected at 10 Hz using a Chem-Clamp (Dagan Corporation) and DEMON Voltammetry and Analysis software (Yorgason et al., 2011). Dopamine release was evoked by single electrical stimulations delivered every 3 min (5 min for cocaine experiments). For experiments comparing dopamine release between compartments, the carbon fiber was placed in the matrix or striosome compartment 300 μm from the stimulating electrode. The stimulation intensity used for each experiment was selected to yield a transient peak approximately 40%–60% of the maximum transient peak (as determined from a preliminary input-output curve for each slice once stable transients were obtained but before baseline measurements) and ranged from 100 to 300 μA. Once five consecutive stable responses were collected (<10% variation in transient peak), experiments would begin. Four to five baseline measurements in the first compartment were collected and then the carbon fiber electrode was moved to the complementary compartment at the same distance from the stimulating electrode. Another four or five responses were collected in the second compartment at the same stimulation intensity as the first compartment. The order of starting compartment was counterbalanced between slices for all experiments. Dopamine transient decay rates, tau, were calculated from an exponential fit encompassing the dopamine transient peak and return to baseline with the data analysis module in DEMON. For experiments examining drug effects between compartments, data were normalized to their respective baseline/pre-drug periods. For modeling of dopamine transient kinetics (V_{max} and apparent K_m) in the cocaine experiments, a Michaelis-Menten-based kinetic modeling module (based on Wu et al. (2001)) in DEMON was used. Briefly, dopamine release