Please cite this article in press as: Ehlinger DG et al. Nicotine-induced and D1-receptor-dependent dendritic remodeling in a subset of dorsolateral striatum medium spiny neurons. Neuroscience (2017), http://dx.doi.org/10.1016/j.neuroscience.2017.05.036

Neuroscience xxx (2017) xxx–xxx

NICOTINE-INDUCED AND D1-RECEPTOR-DEPENDENT DENDRITIC REMODELING IN A SUBSET OF DORSOLATERAL STRIATUM MEDIUM SPINY NEURONS

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Abstract—Nicotine is one of the most addictive substances known, targeting multiple memory systems, including the ventral and dorsal striatum. One form of neuroplasticity commonly associated with nicotine is dendrite remodeling. Nicotine-induced dendritic remodeling of ventral striatal medium spiny neurons (MSNs) is well-documented. Whether MSN dendrites in the dorsal striatum undergo a similar pattern of nicotine-induced structural remodeling is unknown. A morphometric analysis of Golgi-stained MSNs in rat revealed a natural asymmetry in dendritic morphology across the mediolateral axis, with larger, more complex MSNs found in the dorsolateral striatum (DLS). Chronic nicotine produced a lasting (21 day) expansion in the dendritic complexity of MSNs in the DLS, but not dorsomedial striatum (DMS). Given prior evidence that MSN subtypes can be distinguished based on dendritic morphology, MSNs were segregated into morphological subpopulations based on the number of primary dendrites. Analysis of these subpopulations revealed that DLS MSNs with more primary dendrites were selectively remodeled by chronic nicotine exposure and remodeling was specific to the distal-most portions of the dendritic arbor. Co-administration of the dopamine D1 receptor (D1R) antagonist SCH23390 completely reversed the selective effects of nicotine on DLS MSN dendrite morphology, supporting a causal role for dopamine signaling at D1 receptors in nicotine-induced dendrite restructuring. Considering the functional importance of the DLS in shaping and expressing habitual behavior, these data support a model in which nicotine induces persistent and selective changes in the circuit connectivity of the DLS that may promote and sustain addiction-related behavior.

Key words: addiction, habit, direct pathway, indirect pathway, plasticity, adolescence.

INTRODUCTION

Nicotine is a potent reinforcing stimulus, making it among the most addictive substances known (Pontieri et al., 1996). The progression from casual to compulsive drug use is thought to be mediated by mechanisms of neuronal plasticity that underlie normative learning and memory processes (Hyman, 2005). Nicotine targets multiple memory systems associated with reward learning, including the striatum (Rice and Cragg, 2004). The striatum is positioned at the center of cortico-basal ganglia loops that integrate a wide range of input necessary for reinforcement learning, decision-making and motor control (Graybiel, 2000). The striatum is broadly defined along the dorsal and ventral axis (Voorn et al., 2004), with the ventral striatum being the most intensely studied striatal region in the context of reinforcement learning and addiction (Everitt and Robbins, 2005). However, the dorsal striatum also critically participates in reinforcement learning, including decision-making related to action selection (Balleine et al., 2007).

While the ventral striatum is an established target for neuroadaptations in response to nicotine (Koob and Volkow, 2010), nicotine also induces neuroplasticity in the dorsal striatum (Valjent et al., 2004; Pascual et al., 2009; Ortega et al., 2013; Clemens et al., 2014). The dorsal striatum is anatomically and functionally segregated into medial and lateral zones. During the course of instrumental learning, one model suggests the acquisition of goal-directed action selection initially mediated by the DMS is gradually taken over by the dorsolateral striatum (DLS) and expressed as habits (Yin et al., 2004, 2006; Balleine and O'Doherty, 2010). As addiction has been conceptualized as a transition from voluntary consumption to compulsive habit, with a loss of control over drug intake in the face of negative consequences (Belin et al., 2009), a shift in neuronal control from DMS to DLS could underlie the progression from voluntary to habitual drug intake (Yin et al., 2004; Corbit et al., 2012; Gremel and Lovinger, 2016).

One exceptionally persistent form of neuroplasticity commonly associated with addictive drug exposure is dendritic remodeling (Robinson and Kolb, 2004).

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Abbreviations: BAC, bacterial artificial chromosome; D1R, dopamine D1 receptor; DLS, dorsolateral striatum; DMS, dorsomedial striatum; MSNs, medium spiny neurons; PCA, principal components analysis.

http://dx.doi.org/10.1016/j.neuroscience.2017.05.036
0306-4522/

Nicotine-induced dendritic remodeling in the ventral striatum is well-documented (Brown and Kolb, 2001; Hamilton and Kolb, 2005) and is particularly pronounced when exposure occurs during adolescence (McDonald et al., 2007). Furthermore, systemic blockade of dopamine 1 (D1) receptors during nicotine exposure in the adolescent brain completely blocks nicotine-induced dendrite remodeling in the ventral striatum (Ehlinger et al., 2016), suggesting a causal role for dopaminergic signaling at D1 receptors in nicotine-induced dendritic plasticity in the ventral striatum. Comparable measures in the dorsal striatum are lacking. To address this research gap, the dendritic morphology of Golgi-stained medium spiny neurons (MSNs) in the DMS and DLS were completely reconstructed in three-dimensions and morphometrically analyzed after a chronic systemic intermittent nicotine regimen (subcutaneous 0.5 mg/kg, 2 weeks, 8 total injections) during adolescence (PN28-42) in male Sprague-Dawley rats, with or without, co-administration of the highly selective D1 receptor antagonist SCH23390 (subcutaneous 0.05 mg/kg). Because addiction is also defined by chronic relapse (NIDA, 2014), it is important to identify long-lasting changes in cellular plasticity following drug exposure (Grueter et al., 2012), therefore dendritic morphology was measured at a protracted time frame (21-days) following the end of nicotine exposure.

Striatal MSNs can be divided into distinct subpopulations based on anatomical connectivity (i.e., striatonigral "direct" and striatopallidal "indirect" pathways), molecular composition (i.e., D1- and D2-expressing) and functionality (Kreitzer and Malenka, 2008; Kravitz et al., 2012). Recent evidence suggests that striatal MSNs can also be divided into distinct *morphological* subpopulations (Gertler et al., 2008; Gagnon et al., 2017). Whether nicotine selectively influences the dendritic branching pattern of these morphologically defined MSN subpopulations has not been analyzed. MSNs were segregated into subpopulations based on the number of primary dendrites (first order dendrites emanating from the soma). The use of primary dendrites as a criterion structural feature for segregating MSN subpopulations is advantageous in the context of nicotine exposure, as nicotine does not influence this particular feature of MSN morphology (Brown and Kolb, 2001; McDonald et al., 2005, 2007; Hamilton and Kolb, 2005; Ehlinger et al., 2012) and primary dendrite number has been shown previously to differentiate striatal MSN cell types (Gertler et al., 2008). DMS and DLS MSN dendritic remodeling in response to nicotine with or without D1 antagonist co-administration was characterized within morphologically subdivided "large" and "small" subpopulations, based on primary dendrite number.

Our results reveal (1) a naturally existing asymmetry in MSN dendrite morphology between the DLS and DMS, (2) a lasting (at least 21 days) increase in the dendritic complexity of MSNs in the DLS, but not DMS, following chronic nicotine exposure, (3) selective dendritic remodeling for a morphological distinct DLS MSN subpopulation that contains more primary dendrites (large subpopulation), and (4) a blockade of this structural plasticity when animals are co-administered the D1 antagonist SCH23390 during nicotine exposure. Collectively, these results suggest a selective, persistent, and D1 receptor-dependent influence of chronic nicotine on a morphologically discrete DLS MSN subpopulation.

EXPERIMENTAL PROCEDURES

Animals

All data analyzed in this study were derived from tissue generated using experimental procedures that were described in detail in a previously published study (Ehlinger et al., 2016). Male Sprague-Dawley rats (N = 32) (Harlan, IN, USA) arrived to the vivarium at PN21, were housed in groups (n = 3–4) in standard caging, and allowed *ad libitum* access to food and water. The vivarium was controlled for temperature, humidity and light cycle. All experimental procedures were carried out in accordance with the National Research Council Guide for the Care and Use of Laboratory Animals and the George Mason University IACUC. Disclosure of housing and husbandry procedures was in accordance with recommendations for standard experimental reporting in behavioral neuroscience research (Prager et al., 2011).

Drugs and injection schedule

(-)-Nicotine hydrogen tartrate (Nicotine; Sigma Aldrich, St. Louis, MO, USA) was dissolved in 0.9% saline and administered at a dose of 0.5 mg/kg. R(+)-SCH-23390 hydrochloride (SCH-23390; Sigma Aldrich, St. Louis, MO, USA) was dissolved in 0.9% saline and administered at a dose of 0.05 mg/kg. Physiological saline (0.9% NaCl) was the vehicle control. All drugs were administered subcutaneously at a volume of 1 mL/kg. Drugs were administered during adolescence (PN28-42) in rats that were randomly assigned to one of four pretreatment-treatment groups (n = 8 per group): (1) vehicle-vehicle, (2) SCH-23390-vehicle, (3) vehicle-nicotine or (4) SCH-23390-nicotine. The pretreatment drug (vehicle or SCH-23390) was administered exactly 20 min prior to the treatment drug (vehicle or nicotine). Animals were dosed intermittently in their home-cage every other day during an adolescent (PN28-42) timeframe (eight total injections). Rats were grouped housed throughout the course of the study, eliminating isolation-induced stress effects.

Golgi-stain

Prior to Golgi staining there were 21 drug-free days (PN42-63). On PN63, rats were anesthetized with a ketamine/xylazine cocktail and then perfused intracardially with 0.9% saline. The whole brain was placed into a Golgi solution (mercuric chloride, potassium chromate, and potassium dichromate) and stored in the dark at room temperature for 14 days (Golgi solution refreshed after two days). Brains were then transferred into a 30% sucrose solution for three days prior to sectioning. Brains were sectioned (200 μm; coronal) on a vibratome and placed onto gelatinized

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