Neuroscience 274 (2014) 409–418

CORTICOFUGAL PROJECTIONS FROM MEDIAL PRIMARY SOMATOSENSORY CORTEX AVOID EPHA7-EXPRESSING NEURONS IN STRIATUM AND THALAMUS

A. X. TAI [a] **AND L. F. KROMER** [a,b]*

[a] *Department of Neuroscience, Georgetown University Medical Center, 3970 Reservoir Road NW, Washington, DC 20007, USA*

[b] *Interdisciplinary Program in Neuroscience, Georgetown University Medical Center, 3970 Reservoir Road NW, Washington, DC 20007, USA*

Abstract—Within the first two postnatal weeks, corticostriatal axons from the primary somatosensory cortex (S1) form topographic projections that organize into characteristic bands of axon terminals in the dorsolateral striatum. Molecules regulating the development of these topographically organized projections are currently unknown. Thus, the present study investigated whether EphA receptor tyrosine kinases, which regulate axonal guidance in the visual system via axon repulsion, could participate in the formation of corticostriatal connections during development. Prior studies indicate that EphA7-expressing striatal neurons are organized into banded compartments resembling the matrisome innervation pattern formed by cortical afferents from the S1 cortex and that ephrin-A5, a known EphA7 ligand, is expressed in a medial (high) to lateral (low) gradient in S1. Thus, we hypothesized that the organization of EphA7-expressing striatal neurons in banded domains provides a repulsive barrier preventing corticostriatal axons containing EphA7-ligands from innervating inappropriate regions of the striatum. To evaluate this, we injected the anterograde tracer, biotinylated dextran amine (BDA), into two locations in medial areas of S1 (the anterior and posterior whisker fields), which are reported to express high levels of ephrin-A5 during development. Injections were made in mouse pups on postnatal day 9 (P9) and the animals were processed for immunohistochemistry on P12. Our data demonstrate that projections from both the forelimb/anterior whisker field and the posterior whisker field avoid EphA7-expressing neurons and terminate in a banded pattern in regions with very low EphA7-expression. We also determined that corticothalamic projections from medial S1 also exhibit a restricted distribution in the thalamus and avoid neurons expressing EphA7. Thus, our results support the hypothesis that the anatomical organization of striatal and thalamic neurons expressing EphA7 receptors restricts the topographic distribution of cortical afferents from medial regions of S1 which express high levels of ephrin-A5.

Key words: matrix, matrisome, anatomy, ephrin, corticostriatal, corticothalamic.

INTRODUCTION

As the primary input nucleus of the basal ganglia, the striatum plays a central role in regulating cognitive, sensory, motor and limbic modalities. Its primary function is to receive and process basal ganglia-directed activity (a major component of which is from the cortex) and relay it to basal ganglia output nuclei: the globus pallidus and substantia nigra. These nuclei then modulate various modalities directly through inhibitory projections to thalamic and brainstem nuclei (Bolam et al., 2000). Medium spiny neurons (MSNs), the output neurons of the striatum and the main targets for glutaminergic input from the cortex (Gerfen, 2004), are organized into distinct biochemical compartments: the island-like striosomes, which express μ-opioid receptors (Graybiel and Ragsdale, 1978; Herkenham and Pert, 1981), and the surrounding matrix, which expresses EphA4 and EphB1 (Martone et al., 1997; Janis et al., 1999; Richards et al., 2007; Tai et al., 2013). Data indicate that different populations of cortical neurons target the striosomes versus the matrix suggesting the striosomes and matrix possess different molecular signatures (Gerfen, 1984, 1989; Crittenden and Graybiel, 2011). Additional studies also indicate that the matrix compartment itself is comprised of several topographically organized subpopulations of neurons that receive unique cortical inputs (Alloway et al., 1998, 1999; Brown et al., 1998; Hoffer and Alloway, 2001; Hoffer et al., 2005). Since striatal function is highly dependent upon the topographical organization of its neurons and afferents (Voorn et al., 2004), the formation of these complex and selective corticostriatal connections is crucial to proper striatal function.

Corticostriatal projections arrive in the striatum shortly after birth but do not achieve their mature topographic organization until the end of the second postnatal week

*Correspondence to: L. F. Kromer, Department of Neuroscience, Georgetown University Medical Center, Room EG11, The Research Building, 3970 Reservoir Road NW, Washington, DC 20057, USA. Tel: +1-202-687-1827; fax: +1-202-687-0617.
E-mail addresses: axt2@georgetown.edu (A. X. Tai), kromerl@georgetown.edu (L. F. Kromer).

Abbreviations: BDA, biotinylated dextran amine; CO, cytochrome oxidase; DAB, diaminobenzidine; ec, external capsule; P, postnatal day; PBS, phosphate-buffered saline; POm, medial posterior complex of the thalamus; S1, primary somatosensory cortex; VB, ventrobasal complex of the thalamus.

http://dx.doi.org/10.1016/j.neuroscience.2014.05.039
0306-4522/

(Iniguez et al., 1990; Christensen et al., 1999; Jain et al., 2001; Sohur et al., 2014). Several molecules, such as netrin-1 and semaphorins, are reported to regulate the axonal outgrowth of corticofugal axons and their guidance within the internal capsule to their target brain regions in the ventral forebrain and thalamus (Grant et al., 2012). However, the molecules that regulate the topographic organization of corticostriatal terminal fields within the striatum have not been identified. One family of axon guidance molecules that could possibly regulate this process are the Eph family of receptor tyrosine kinases and their membrane bound ligands, ephrins. During development these molecules guide axons and regulate the formation of topographic projections in multiple brain regions, primarily through repulsive interactions (Flanagan and Vanderhaeghen, 1998; O'Leary and Wilkinson, 1999; Pasquale, 2005; Boyd et al., 2014). These repulsive mechanisms can be mediated either via "forward" signaling through the EphA receptor or via "reverse" signaling through the corresponding ephrin (Holland et al., 1996; Feldheim et al., 1998; Chilton, 2006; Feldheim and O'Leary, 2010). Recent studies indicate that cortical afferents enter the striatum postnatally (Sohur et al., 2014), after EphA7-expressing matrisome neurons have organized into their characteristic banded distribution within the matrix compartment of the striatum (Janis et al., 1999; Tai et al., 2013). Interestingly, the banded domains of EphA7 + striatal neurons resemble the reported topographic distribution of corticostriatal axons from the primary somatosensory cortex (S1), which also terminate in banded domains within the dorsolateral striatum (Brown et al., 1998; Alloway et al., 1999, 2009; Hoffer and Alloway, 2001). Molecular studies indicate that corticostriatal and corticothalamic neurons located in layers V and VI of the medial S1 barrel cortex express high levels of ephrin-A5 perinatally, and that these neurons are segregated from adjacent cortical areas containing EphA7-expressing neurons (Vanderhaeghen et al., 2000; Miller et al., 2006; Dye et al., 2011a,b). Ephrin-A5 exhibits a high binding affinity to EphA7 (Himanen et al., 2004) and this ligand–receptor pair was recently implicated in the guidance of corticothalamic projections to various thalamic nuclei via a repulsive mechanism (Torii and Levitt, 2005; Torii et al., 2013). Thus, we hypothesize that repulsive interactions between ephrin-As, such as ephrin-A5, on cortical axons and EphA7 receptors present on a subset of striatal neurons also may serve to guide the topographic distribution of corticostriatal axons.

To evaluate this hypothesis, we injected the anterograde axonal tracer, biotinylated dextran amine (BDA) into medial regions of the S1 cortex where neurons in layer V and VI express high levels of ephrin-A5 perinatally (Vanderhaeghen et al., 2000; Miller et al., 2006; Dye et al., 2011a,b) and evaluated the organization of the labeled corticostriatal terminal arbors within the striatum. In addition, we also evaluated whether corticothalamic afferents from the medial S1 barrel cortex avoid regions of the ventrobasal (VB) and medial posterior complex (POm) of the thalamus which contain neurons expressing EphA7 receptors.

EXPERIMENTAL PROCEDURES

Animals

All animal experiments strictly conformed to guidelines set forth by the National Institutes of Health (NIH) and the Georgetown University Institutional Animal Care and Use Committee. Mice for these studies were on a mixed Swiss-Webster:C57BL-6:129:FVB/N background and were bred at Georgetown University as part of a transgenic mouse colony. Founder mice for this colony were obtained from the MMRRC and Dr. David Feldheim (UC, Santa Cruz).

BDA injections

Mice were postnatal day 9 (P9) at the time of surgery. Prior to surgery, each mouse pup was anesthetized with intraperitoneal injections of a cocktail containing ketamine (10 mg/ml), acepromazine (1 mg/ml) and xylazine (10 mg/ml) in sterile saline with dosing based on their weight (0.01 ml/1 g). After anesthesia induction, the skin over the cranium was cleaned with antiseptic scrub and resected, and the animal was placed in a mouse stereotaxic instrument. Approximately 50 nl of 10% BDA solution (Molecular Probes, D-7135, Eugene, OR) was injected into anterior or posterior regions of the right medial S1 barrel cortex with a glass pipette coupled to a Hamilton microsyringe (Reno, NV). Injection coordinates were based on the Atlas of the Developing Mouse Brain by Paxinos & Franklin (Paxinos and Franklin, 2001). Following tracer injections, the wound margin was closed with LiquiVet (Oasis Medical, Mettawa, IL). After recovery from anesthesia, the pups were returned to their mothers and survived for 3 days postinjection to allow transport of the injected BDA to axonal terminals in the striatum before the animals were prepared for histology.

Histochemistry

For immunohistochemical staining procedures, P12 mouse pups were deeply anesthetized with a cocktail containing ketamine, acepromazine, and xylazine, perfused transcardially with 0.1 M phosphate buffer (pH 7.2–7.4), followed with 4% paraformaldehyde in 0.1 M phosphate buffer. After perfusion, the brain was removed, postfixed for 3 h in 4% buffered paraformaldehyde (4 °C), and then cryoprotected in 20% (w/v) sucrose in 0.1 M phosphate buffer for 24–72 h at 4 °C. The brains were then frozen in Tissue Tek (Sakura Finetek, Torrance, CA), stored at −80 °C until sections (20 μm) were cut on a cryostat and mounted on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA). Mounted sections were stored at −20 °C to −80 °C until they were processed for histology.

EphA7 immunohistochemistry. Antigen retrieval was used to obtain optimal detection of EphA7 receptor protein expression in tissue sections. For this procedure mounted brain sections were autoclaved for 2 min in