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Research Report

Quantification of synaptic density in corticostriatal projections from rat medial agranular cortex

Roger L. Reep[a,*], *Jennifer H. Wu*[a], *Joseph L. Cheatwood*[b,c], *James V. Corwin*[d], *Gwendolyn L. Kartje*[b,c], *Anis Mir*[e]

[a]*Department of Physiological Sciences and McKnight Brain Institute, University of Florida, Box 100144, Gainesville, FL 32610, USA*
[b]*Department of Neurology, Stritch School of Medicine, Loyola University, USA*
[c]*Neurology Service, Hines Veterans Affairs Hospital, USA*
[d]*Department of Psychology, Northern Illinois University, USA*
[e]*Neuroscience Research, Novartis Institutes for BioMedical Research, Basel, Switzerland*

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ABSTRACT

Medial agranular cortex (AGm) has a prominent bilateral projection to the dorsocentral striatum (DCS). We wished to develop a normal baseline by which to assess neuronal plasticity in this corticostriatal system in rats with neglect resulting from a unilateral lesion in AGm, followed by treatment with agents that promote sprouting and functional recovery in other systems. Injections of biotinylated dextran amine were made into AGm in normal rats, and unbiased sampling was used to quantify the density of axons and axonal varicosities present in DCS (the latter represent presynaptic profiles). Labeling density in contralateral DCS is approximately half of that seen in ipsilateral DCS (this ratio is 0.50 for axons, 0.55 for varicosities). The ratio of varicosities is stable over a greater than seven-fold range of absolute densities. There is no consistent relationship between the absolute density of axons and axon varicosities; however, the ratio measures are strongly correlated. We conclude that changes in the contralateral/ipsilateral ratio of axon density after experimental treatments do reflect changes in synaptic density, but axon varicosities are likely to be the most sensitive anatomical parameter by which to assess plasticity at the light microscopic level.

1. Introduction

Corticostriatal projections have been known to exist since Cajal (1891 — see Webster, 1961). However, the existence of bilateral corticostriatal projections from single cortical areas was discovered more recently, in the 1960s (see Carman et al., 1965). Due to the presence of bilateral corticostriatal projections in a number of species which have been studied (Kemp and Powell, 1970; Fallon and Ziegler, 1979; Cospito and Kultas-Ilinsky, 1981; Royce, 1982; Fisher et al., 1984; Wilson, 1986), these projections are considered to be a feature typical of mammalian brains.

In rats, medial agranular cortex (area AGm, or Fr2) is the source of a particularly prominent projection to the dorsocentral striatum (DCS) (Reep et al., 1987; Wilson, 1987; Reep and Corwin, 1999; Reep et al., 2003). Two classes of cortical neurons contribute to this projection system. The majority of the projection originates from pyramidal neurons in layer Va and

* *Corresponding author*. Fax: +1 352 392 5145.
E-mail address: reep@mbi.ufl.edu (R.L. Reep).

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layer III, whereas a much smaller portion originates as collaterals from deeper layer Vb/VI pyramidal cells that project to the thalamus and brainstem (Wilson, 1987; Cowan and Wilson, 1994).

Recent behavioral studies have pointed to the importance of AGm and DCS as components of a cortical–subcortical network subserving directed spatial attention in rats (Burcham et al., 1997; Corwin and Reep, 1998; Reep et al., 2004). Unilateral lesions of AGm or DCS result in severe multimodal neglect of visual, auditory, and tactile stimuli presented contralaterally that is qualitatively similar to neglect in primates (Crowne and Pathria, 1982; Corwin et al., 1986; Van Vleet et al., 2002), and recent studies indicate that the integrity of DCS is essential for recovery of function from lesion-induced attentional disorders. Axon-sparing lesions of DCS produce severe multimodal neglect of stimuli presented contralesionally, whereas lesions placed more laterally and ventrally do not (Van Vleet et al., 2002). The dopamine agonist apomorphine induces acute recovery in animals with severe neglect produced by cortical lesions (Corwin et al., 1986, 1996; King and Corwin, 1990), and in these cases direct infusion of apomorphine into DCS results in dose-dependent acute recovery (Van Vleet et al., 2003). However, apomorphine does not result in recovery when administered to animals with lesions of DCS (Van Vleet et al., 2000), indicating that DCS is not only an essential component of the circuitry for neglect, but also that its integrity is necessary for recovery. Vargo and Marshall (1996a,b) were the first to suggest that sprouting in the dorsolateral quadrant of the striatum, which includes the DCS, was correlated with neglect and behavioral recovery from AGm-induced neglect. They found that neglect was correlated with decreases in NMDA and kainate receptors in the ipsilesional dorsolateral striatum, and recovery was correlated with a normalization of kainate receptors and a 10% increase in NMDA receptors in this region.

Although the recovery observed by Vargo et al. could be based solely on changes at the receptor level, recent studies following lesions of motor cortex have found that loss of corticostriatal input can result in sprouting from the contralesional homotopic cortex, and functional recovery (Napieralski et al., 1996; Cheng et al., 1997, 1998; McNeill et al., 1999; Meshul et al., 2000). These finding suggest the intriguing possibility that sprouting from the contralesional AGm may be the basis for the receptor changes found by Vargo and Marshall in the dorsolateral striatum. Therefore, DCS has become the focus for efforts to induce plasticity and long-term functional recovery from neglect in the rat model, and to determine the neurological basis for recovery.

Changes in the density of corticostriatal projections have been used as a measure of neuronal plasticity following cortical lesions and subsequent treatments intended to induce neuronal sprouting and functional recovery. Several studies have assessed treatment-related alterations in the ratio of contralateral to ipsilateral (contra/ipsi) axonal labeling following a cortical injection of the anterograde tracer BDA (Napieralski et al., 1996; Kartje et al., 1999; Carmichael and Chesselet, 2002; Riban and Chesselet, 2006). In normal and sham-operated animals, quantification of axonal density revealed mean contra/ipsi ratios of 0.20–0.26 in dorsolateral striatum following tracer injection in sensorimotor cortex (including areas AGl, FL, and HL) (Kartje et al., 1999; Carmichael and Chesselet, 2002), and a mean ratio of 0.62 in dorsomedial striatum (Kartje et al., 1999). A similar study in mice produced a mean contra/ipsi axon density ratio of 0.37 in dorsolateral striatum (Riban and Chesselet, 2006). One of the advantages of using the contra/ipsi ratio is that it controls for variation in the size and density of BDA injection sites. This is a variable which can have a significant influence on the absolute amount of labeling seen, particularly in the corticostriatal projection, which consists largely of small caliber collaterals that can be difficult to fill completely. One limitation of these studies is that changes in axon density are at best an indirect measure of changes in synaptic density which are likely to be the actual basis for functional recovery.

We wished to develop a normal baseline by which to assess sprouting in DCS in rats with neglect resulting from a unilateral lesion in AGm, followed by treatment with agents that promote sprouting and functional recovery in other systems. Our goal was to develop a method to quantify synaptic density at the light microscopic level, based on the fact that axon varicosities represent presynaptic profiles in a

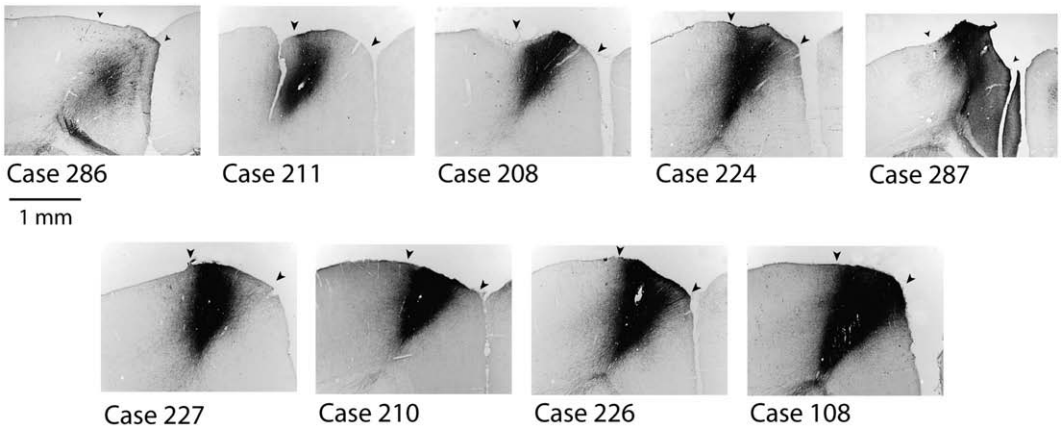

Fig. 1 – Nine cases of BDA injection sites in AGm, arranged in order of increasing size and density. The center of each injection site is shown at equivalent magnification and illumination. All injections were confined to AGm and centered at a–p +0.8. Arrowheads indicate boundaries of AGm determined from adjacent Nissl-stained sections.