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Cytoarchitecture and cortical connections of the anterior insula and adjacent frontal motor fields in the rhesus monkey

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ABSTRACT

The cytoarchitecture and cortical connections of the ventral motor region are investigated using Nissl, and NeuN staining methods and the fluorescent retrograde tract tracing technique in the rhesus monkey. On the basis of gradual laminar differentiation, it is shown that the ventral motor region stems from the ventral proisocortical area (anterior insula and dorsal Sylvian opercular region). The cytoarchitecture of the ventral motor region is shown to progress in three lines, as we have recently shown for the dorsal motor region. Namely, root (anterior insular and dorsal Sylvian opercular area ProM), belt (ventral premotor cortex) and core (precentral motor cortex) lines. This stepwise architectonic organization is supported by the overall patterns of corticocortical connections. Areas in each line are sequentially interconnected (intralineal connections) and all lines are interconnected (interlinear connections). Moreover, root areas, as well as some of the belt areas of the ventral and dorsal trend are interconnected. The ventral motor region is also connected with the ventral somatosensory areas in a topographic manner. The root and belt areas of ventral motor region are connected with paralimbic, multimodal and prefrontal (outer belt) areas. In contrast, the core area has a comparatively more restricted pattern of corticocortical connections. This architectonic and connectional organization is consistent in part, with the functional organization of the ventral motor region as reported in behavioral and neuroimaging studies which include the mediation of facial expression and emotion, communication, phonic articulation, and language in human.

Abbreviations: A, arm; ac, anterior commissure; amts, anterior medial temporal sulcus; amy, amygdala; apos, anterior parieto-occipital sulcus; as, arcuate spur; Ca, caudate nucleus; CA1, Cornu Ammonis 1 subfield according to Lorente de Nó (1934); cc, corpus callosum; ccg, genu of the corpus callosum; ccs, splenium of the corpus callosum; cf, calcarine fissure; cl, claustrum; cgs, cingulate sulcus; cs, central sulcus; D, digit; DY, diamidino yellow; F, face; FB, fast blue; FRT, fluorescent retrograde tracer; gp, globus pallidus; hf, hippocampal fissure; hp, hippocampus; hy, hypothalamus; Iag, insula, agranular sector; ic, internal capsule; Idg, insula, dysgranular sector; Ig, insula, granular sector; ilas, inferior limb of the arcuate sulcus; ios, inferior occipital sulcus; Ipro, insula proisocortex; iprs, inferior precentral sulcus; ips, intraparietal sulcus; L, leg; lf, lateral fissure; los, lateral orbital sulcus; ls, lunate sulcus; MI, primary motor cortex (area 4); MII, supplementary motor cortex (area 6 m); M3, rostral cingulate motor cortex (areas 24c and 24d); M4, caudal cingulate motor cortex (areas 23c and 23d); mos, medial orbital sulcus; oc, optic chiasm; OFC, orbitofrontal cortex; olf, olfactory sulcus; ot, optic tract; ots, occipital temporal sulcus; paAc, para-auditory cortex caudal; paAlt, para-auditory cortex lateral; par, para-auditory cortex rostral; pal, parainsular cortex; pAmC, peri-amygdaloid cortex; ParaSub, parasubiculum Pmts, posterior middle temporal sulcus; POC, primary olfactory cortex; poms, medial parieto-occipital sulcus; preSMA, pre-supplementary motor cortex; PreSub, presubiculum; Pro, proisocortex; proA, proauditory cortex; ProM, proisocortical motor cortex; ProStr, area prostriata; ProSub, prosubiculum; ps, principal sulcus; pu, putamen; reit, retroinsular temporal area; reipt, retroinsular parietal temporal area; rf, rhinal fissure; RI, retroinsular area; ros, rostral sulcus; SI, primary somatosensory cortex; SII, secondary somatosensory cortex; sbps, subparietal sulcus; slas, superior limb of the arcuate sulcus; spcs, superior precentral sulcus; spocs, superior postcentral sulcus; SSA, supplementary somatosensory cortex (area PEci); STG, superior temporal gyrus; stn, subthalamic nucleus; sts, superior temporal sulcus; Sub, subiculum; th, thalamus; TMA, transitional motor area; Tpro, temporal proisocoretex; TSA, transitional sensory area; v, ventricle.

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1. Introduction

In a recent study, we have shown that the dorsal motor and premotor regions are related architectonically and connectionally with the rostral cingulate proisocortical areas and the cingulate motor regions in a stepwise manner (Morecraft et al., 2012). Moreover, it was shown that the dorsal motor, premotor and cingulate motor regions can be grouped in a tripartite manner by virtue of their interconnections. While studying the architectonics and connections of the ventral somatosensory areas, it was shown that these areas are sequentially related to the ventral motor, ventral premotor and the insular regions (Cipolloni and Pandya, 1999). A great deal of interest has also been focused on the ventral precentral, premotor and prefrontal areas using neuroanatomical, electrophysiological, behavioral, and neuroimaging methodologies (e.g., Goldschlak et al., 1984; Matelli et al., 1986; Barbas and Pandya 1987, 1989; Rizzolatti et al., 1987, 2014; Gentilucci et al., 1989; Murray and Sessle, 1992; Morecraft et al., 2001, 2014; Petrides and Pandya, 2002; Simonyan and Jürgens, 2002a,b; 2005; Wang et al., 2002; Hatanaka et al., 2005; Jürgens and Ehrenreich, 2007; Frey et al., 2008, 2014; Gerbella et al., 2010, 2011, 2015; Umarova et al., 2010; Arce et al., 2013; Margulies and Petrides, 2013; Bonini et al., 2014). This has largely been due to the important role that this brain region has in communication, articulation and orofacial movements including facial expression.

In the present study, we have investigated the hypothesis that, unlike the dorsal motor and premotor areas which have been shown to be related to the anterior cingulate proisocortex (Morecraft et al., 2012), the ventral motor and premotor areas may have a closer relationship with the ventral proisocortical areas, that is, area ProM (PrCo) and rostral insula. Also, we investigated the possibility that the ventral motor and premotor areas are organized into a tripartite manner, that is, into a root, belt and core pattern much like we have found for the dorsal motor and premotor areas (Morecraft et al., 2012). We therefore have re-examined the architecture and connections of the ventral proisocortical, as well as the ventral motor and premotor areas using Nissl and NeuN stained material and the fluorescent retrograde tract tracer technique respectively. As will be demonstrated, our study indicates that the ventral motor and premotor areas are structurally related to the insular cortex in a stepwise manner, and like the dorsal motor and premotor areas, are also organized in a tripartate manner.

2. Material and methods

Cortical architecture and connections of the ventral motor and premotor areas as well as the rostral Sylvian opercular and insular cortices were investigated in 10 rhesus monkeys (*Macaca mulatta*). Five monkeys were used to investigate the pattern of cortical cytoarchitecture, and 5 animals were used to study the corticocortical connections (9 different injection sites). All surgical and experimental procedures were approved by the Institutional Animal Care and Use Committee at the University of South Dakota and followed the guidelines for the ethical treatment of animals outlined by the United States Department of Agriculture (USDA) and the National Institutes of Health. All monkeys were housed and cared for in a USDA and Association for Assessment and Accreditation of Laboratory Animal Care approved and inspected facility. Fluorescent retrograde tracing (FRT) injections were used in multiple experimental combinations in this study to minimize the number of animals needed to accomplish the study aims and strengthen our understanding of the spatial relationships of the potentially different cortical connectional patterns in the same experimental brain. Thus, in 4 of the 5 animals used for our connectional evaluation, we injected both diamidino-yellow (DY) and Fast Blue (FB) into different targets of the same cortical hemisphere. These paired experiments included injection Cases 1 and 2 (SDM66), Cases 3 and 4 (SDM69), Cases 5 and 7 (SDM40) and Cases 6 and 8 (SDM39). The surgical and experimental procedures for the 3 animals processed for Nissl substance at Boston University were approved by the Boston University Institutional Animal Care and Use Committee.

2.1. Neurosurgical and cortical injection procedures

All surgical procedures were performed using sterile methods and have been described in detail (Morecraft et al., 2012). Breifly, each monkey was immobilized with atrophine (0.5 mg/kg) and ketamine hydrochloride (10 mg/kg). The animals were then intubated and anesthetized with isofluorane inhalation (1.5–2% with surgical grade air/oxygen) except for Case 8, which was anesthetized with pentobarbitol. The monkey was then placed into a head holding device and then Mannitol (1.0–1.5 g/kg) was administered intravenously by infusion line drip.

A skin incision and frontoparietal craniotomy was made over the lateral surface of the cerebral hemisphere. The lateral cortical surface of interest was then exposed by making a cruciform or U-shaped dural flap. In the case of neurosurgical exposure of the insula, a fine tipped syringe was used to slowly infuse a 0.9% solution of sterile saline below the arachnoid matter overlying the lateral fissure. This process created a small space, or opening in the lateral fissure. Cottonioid padding was then inserted between the frontal and temporal operculum to increase this space, facilitating clear exposure of the insular cortex in the depths of the lateral fissure.

With the aid of a surgical microscope, an injection of the retrograde neural tracer FB (at a concentration of 3–5% in 0.9% phosphate buffer at pH 7.4) and DY (at a concentration of 3–4% in 0.9% phosphate buffer at pH 7.4) was made into predetermined cortical targets. Pressure injections (0.3–0.4 μL per penetration) of each tracer were made using a Hamilton microsyringe with a cannule tip inserted 2–3 mm below the cortical surface as described previously (Morecraft et al., 2012). Only one penetration was made in the intended cytoarchitectonic region in all Cases with the exception of Case 9 (SDM8), which received 3 closely spaced injections of DY into the physiologically-defined face representation of the primary motor cortex (M1). Following injections of tract tracing compound in all Cases, the dura was closed and the bone flap was replaced and anchored. Subsequently the muscle and skin were closed in layers and the animal monitored postoperatively. Bicillin LA (300,000–750,000 units) was used as prophylaxis antibiotic and buprenorphine (0.01 mg/kg) was used as a post-operative analgesic.

2.2. Intracortical microstimulation procedure

In all experiments, excluding the insula injection Case, the ventral premotor cortex and ventral precentral motor cortex was physiologically mapped using a tungsten electrode (impedance 0.5–1.5 MΩ) as previously described in detail (Morecraft et al., 2012). Briefly, an initial stimulation was attempted 100 μm below the pia and then the electrode was advanced at 500 μm intervals at which the stimulation process was repeated at each depth until movement was clearly detected. Movements were evoked using a train duration of 50 ms and pulse duration of 0.2 ms delivered at 330 Hz (Huntley and Jones 1991; Morecraft et al., 2012, 2013). Current intensity ranged between 1 and 90 μA. Threshold currents were determined and the evoked movements were recorded if noted by at least 2 observers. In all microstimulation experimentals conducted in the present study, orofacial movements were evoked