Neuroscience 147 (2007) 727–745

THALAMIC CONNECTIONS OF THE DORSAL AND VENTRAL PREMOTOR AREAS IN NEW WORLD OWL MONKEYS

I. STEPNIEWSKA,[a]* T. M. PREUSS[b] AND J. H. KAAS[a]

[a]*Department of Psychology, Vanderbilt University, 301 David K. Wilson Hall, 111 21st Avenue South, Nashville, TN 37203, USA*

[b]*Division of Neuroscience and Center for Behavioral Neuroscience, Yerkes National Primate Research Center, Emory University, 945 Gateway Road NE, Atlanta, GA 30329, USA*

Abstract—Thalamic connections of two premotor cortex areas, dorsal (PMD) and ventral (PMV), were revealed in New World owl monkeys by injections of fluorescent dyes or wheat-germ agglutinin conjugated to horseradish peroxidase (WGA-HRP). The injections were placed in the forelimb and eye-movement representations of PMD and in the forelimb representation of PMV as determined by microstimulation mapping. For comparison, injections were also placed in the forelimb representation of primary motor cortex (M1) of two owl monkeys. The results indicate that both PMD and PMV receive dense projections from the ventral lateral (VL) and ventral anterior (VA) thalamus, and sparser projections from the ventromedial (VM), mediodorsal (MD) and intralaminar (IL) nuclei. Labeled neurons in VL were concentrated in the anterior (VLa) and the medial (VLx) nuclei, with only a few labeled cells in the dorsal (VLd) and posterior (VLp) nuclei. In VA, labeled neurons were concentrated in the parvocellular division (VApc) dorsomedial to VLa. Labeled neurons in MD were concentrated in the most lateral and posterior parts of the nucleus. VApc projected more densely to PMD than PMV, especially to rostral PMD, whereas caudal PMD received stronger projections from neurons in VLx and VLa. VLd projected exclusively to PMD, and not to PMV. In addition, neurons labeled by PMD injections tended to be more dorsal in VL, IL, and MD than those labeled by PMV injections. The results indicate that both premotor areas receive indirect inputs from the cerebellum (via VLx, VLd and IL) and globus pallidus (via VLa, VApc, and MD). Comparisons of thalamic projections to premotor and M1 indicate that both regions receive strong projections from VLx and VLa, with the populations of cells projecting to M1 located more laterally in these nuclei. VApc, VLd, and MD project mainly to premotor areas, while VLp projects mainly to M1. Overall, the thalamic connectivity patterns of premotor cortex in New World owl monkeys are similar to those reported for Old World monkeys.

Key words: motor thalamus, ventral lateral nucleus, motor cortex, primate, frontal cortex.

Judging from microstimulation and other studies on Old World monkeys (Matelli et al., 1985; Huerta et al., 1986; Godschalk et al., 1995; Gabernet et al., 1999; for reviews see Geyer et al., 2000; Donoghue and Sanes, 1994; Luppino and Rizzolatti, 2000; Dum and Strick, 2002), New World monkeys (Gould et al., 1986; Stepniewska et al., 1993, 2006; Preuss et al., 1996), and prosimian galagos (Wu et al., 2000; Fang et al., 2005), with supporting imaging data from humans (Zilles et al., 1995; Geyer et al., 1996; Roland and Zilles, 1996; Fink et al., 1997; Picard and Strick, 2001), all primates have a primary motor area, M1, and dorsal (PMD) and ventral (PMV) premotor areas, sometimes with subdivisions (e.g. rostral and caudal divisions of PMD and PMV in macaques). While the thalamic connections of these three major divisions of motor cortex have been extensively described in macaques (Strick, 1975, 1986; Schell and Strick, 1984; Jones, 1987; Matelli et al., 1989; Darian-Smith et al., 1990a,b; Nakano et al., 1992, 1993; Kurata, 1994; Matelli and Luppino, 1996; Rouiller et al., 1999; Morel et al., 2005), and recently reported in galagos (Fang et al., 2006), only the thalamocortical connections of M1 have been systematically studied in New World monkeys (Stepniewska et al., 1994b). Thus, almost nothing is known about the patterns of thalamic inputs to PMD and PMV of New World monkeys, which constitute a large and varied branch of the primate radiation.

Although the thalamocortical connections of PMD and PMV are not known for humans or apes, a useful premise is to suppose that these connections are similar in all primates, and thus the thalamocortical connections described in macaques reflect those present in the human brain. This premise would be more credible, however, if comparative studies revealed little variation in connection patterns across the primate taxa that are available for study. In contrast, the finding of considerable variation in the connection patterns across primate taxa would suggest that the projection of results from a single primate group, such as macaques, to humans, should be made only with considerable caution. Here, we placed injections of fluo-

*Corresponding author. Tel: +1-615-322-5263 or +1-615-322-7491; fax: +1-615-343-8449.
E-mail address: iwona.stepniewska@vanderbilt.edu (I. Stepniewska).

Abbreviations: AChE, acetylcholinesterase; CL, central lateral nucleus; CM, centromedian nucleus; CO, cytochrome oxidase; DY, Diamidino Yellow; FB, Fast Blue; FR, fluororuby; ICMS, intracortical microstimulation; IL, intralaminar nuclei; MD, medial dorsal nucleus; M1, primary motor area; M1c, primary motor area, caudal subdivision; M1r, primary motor area, rostral subdivision; Pc, paracentral nucleus; PMD, dorsal premotor cortex; PMDc, dorsal premotor cortex, caudal subdivision; PMDr, dorsal premotor cortex, rostral subdivision; PMV, ventral premotor cortex; PMVc, ventral premotor cortex, caudal subdivision; PMVr, ventral premotor cortex, rostral subdivision; SMA, supplementary motor area; VA, ventral anterior nucleus; VAmc, ventral anterior nucleus, medial (magnocellular) subdivision; VApc, ventral anterior nucleus, lateral (parvocellular) subdivision; VL, ventral lateral nucleus; VLa, ventral lateral nucleus, anterior subdivision; VLd, ventral lateral nucleus, dorsal subdivision; VLp, ventral lateral nucleus, principal subdivision; VLx, ventral lateral nucleus, medial subdivision; VM, ventral medial nucleus; WGA-HRP, wheat-germ agglutinin conjugated to horseradish peroxidase.

0306-4522/07$30.00+0.00 © 2007 IBRO. Published by Elsevier Ltd. All rights reserved.
doi:10.1016/j.neuroscience.2007.03.054

rescent dyes or wheat germ agglutinin into areas of motor cortex to label thalamic neurons projecting to PMD and PMV of owl monkeys, New World monkeys where these areas had been previously defined by patterns of movements evoked by microstimulation (Preuss et al., 1996). In addition, some of the cortical connections of PMV and PMD are already known in owl monkeys (Stepniewska et al., 2006) and in New World squirrel monkeys (Dancause et al., 2006a,b). Although the thalamic connections of M1 have been previously reported in owl monkeys (Stepniewska et al., 1994b), we also describe thalamic connections of M1 in two of the present cases, so that the connections of primary motor and premotor cortex can be directly compared in the same cases. The results suggest that the thalamocortical connections of M1, PMV and PMD are highly similar in both New and Old World monkeys, although some differences appear to exist.

EXPERIMENTAL PROCEDURES

Intracortical microstimulation (ICMS) procedures

The thalamic projections to PMD and PMV areas were revealed in five adult owl monkeys (*Aotus trivirgatus*) that were part of our previous investigation of corticocortical connectivity (Stepniewska et al., 2006). Owl monkeys came from the Center for Neotropical Primate Research and Resources at University of South Alabama (Mobile, AL, USA). All procedures were approved by Vanderbilt University Animal Care and Use Committee, and followed the guidelines of the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Our procedures were designed to prevent or minimize pain and distress, and the number of animals used was the minimum necessary to obtain sufficient data for the study. All surgical procedures were carried out under aseptic conditions and are described in detail in Stepniewska et al. (1993) (see also Stepniewska et al., 2006). Tracer injections were placed in the forelimb and eye-movement representations of PMD and in the forelimb representation of PMV, guided by microstimulation mapping and confirmed by subsequent architectonic analysis (Table 1; and see Preuss et al., 1996). Each animal received injections of one to three distinguishable tracers (Table 1), among them wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP; Sigma; 2% in distilled water), and the fluorescent dyes Diamidino Yellow (DY; Sigma, St. Louis, MO, USA; 2% in phosphate buffer), Fast Blue (FB; Sigma; 3% in distilled water), and fluororuby (FR; Molecular Probes, Eugene, OR, USA; 10% in distilled water). For comparison, injections were also placed in the forelimb representation of M1 of two monkeys. Following a survival period of 4–9 days, depending on the tracer used, animals were given a lethal dose of barbiturate and perfused through the heart with buffered physiological saline, followed by 2–4% paraformaldehyde, and then 2–4% paraformaldehyde with 10% sucrose. Brains were removed, and the thalamus was separated from the cortex and immersed in 30% sucrose overnight.

Table 1. Summary of experimental cases

Case	PMD		PMV	M1	
	PMDr	PMDc		M1r	M1c
92-42L		10% FR			
91-72L	10% FR		2% WGA-HRP		
90-55L		2% DY			3% FB
92-83L		2% DY	10% FR	3% FB	
93-4L			2% DY		

Histology and architectonics

Thalamic blocks were frozen-sectioned on a sliding microtome at a thickness of either 40 or 50 μm in the coronal plane. For analysis of the distribution of neurons labeled by the fluorochromes, a series of one in four sections was mounted unstained. Subsequently, these sections were counterstained with Thionin for cytoarchitectonic evaluation. To reveal WGA-HRP in cases with such injections, a one-in-four series of sections was treated with tetramethylbenzidine as the chromogen according to the protocol of Gibson et al. (1984). An additional series of sections was processed for cytochrome oxidase (CO) (Wong-Riley, 1979) and another series for acetylcholinesterase (AChE) (Geneser-Jensen and Blackstad, 1971).

The locations of cells labeled with fluorochromes were charted with a Leitz microscope (Leica Microsystems, Wetzlar, Germany) connected to an X-Y plotter, with 360 nm (for FB, DY) and 530–560 nm (for FR) wavelength excitation filters. Sections with WGA-HRP label were studied under darkfield illumination and the locations of HRP injection sites and labeled neurons were drawn using a drawing tube attached to a Wild macroscope at the same scale as adjacent sections with fluorescent labeling. Both series of sections were then superimposed by using blood vessels and other landmarks identified in the tissue sections as guides for alignment. The thalamic nuclei were delineated using architectonic criteria described briefly here, and in more detail elsewhere (Stepniewska et al., 1994a). The plotted labeled cells were counted for each nucleus across the series of one in four sections through the motor thalamus.

Photomicrographs were made using a 35 mm Olympus C-35 camera mounted on the Olympus BH-2 microscope (Olympus America, Inc., Melville, NY, USA). Negatives were scanned at 300 dots per inch with a Polaroid Sprint Scan 35 scanner (Polaroid Corporation, Cambridge, MA, USA). The digitized images were adjusted for brightness and contrast, cropped and pasted in the frame, where text was added using Photoshop 7.0 software (Adobe Systems Inc., San Jose, CA, USA). Except for contrast adjustment and cropping, the images were not altered in any way.

RESULTS

In our recent publication (Stepniewska et al., 2006), we described the pattern of corticocortical connections in owl monkeys based on the injections of anatomical tracers into different premotor and motor regions guided by ICMS. The goal of the present study was to establish the patterns of thalamic connections to motor regions from the label resulting from the injections in the same cases. The results demonstrate different, but partially overlapping, sets of thalamic connections for motor and premotor cortical areas.

Location of injection sites

The extent and somatotopic organization of premotor cortex in owl monkeys (Fig. 1) has been determined in previous microstimulation and architectonic studies (see Stepniewska et al., 1993; Preuss et al., 1996). Generally, premotor cortex was distinguished from M1 by higher average thresholds for movements evoked by ICMS and by differences in the somatotopic arrangement of evoked movements. PMD represents movements of forelimb, hindlimb, upper trunk, and eyes. The hindlimb and forelimb are represented in the caudal dorsal premotor cortex (PMDc), whereas eye and face movements are repre-