Microstimulation reveals specialized subregions for different complex movements in posterior parietal cortex of prosimian galagos

Iwona Stepniewska, Pei-Chun Fang, and Jon H. Kaas*

Department of Psychology, Vanderbilt University, Nashville, TN 37203

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Posterior parietal cortex of prosimian galagos consists of a caudal half characterized by connections with visual cortex and a rostral half connected with motor, premotor, and visuomotor areas of frontal cortex. When 500-ms trains of electrical pulses were used to stimulate microelectrode sites throughout posterior parietal cortex, movements were elicited only from the rostral half. The movement zone reflected an overall pattern of somatotopy, from eye and face movements most ventrally to hindlimb movements most dorsally. In addition, subregions or zones of this movement cortex seemed to be devoted to components of different, ethologically significant behaviors. Thus, microstimulation within separate zones of cortex elicited reaching, hand-to-mouth, defensive, or aggressive movements. The finding of similar classes of elicited movement patterns from frontal and more recently intraparietal cortex of macaques suggests that multiareal circuits for biologically significant behaviors are components of all primate brains and that these circuits can be activated by long trains of current pulses at rostral locations in posterior parietal cortex.

cortical connections | intraparietal cortex | motor cortex | visual cortex

The results of recent studies using microelectrodes to stimulate cortex in macaque monkeys have raised the possibility that motor, premotor, and intraparietal regions of cortex are functionally divided into several cortical zones specialized for mediating different, ethologically relevant behaviors (1–7). Within precentral motor, and premotor cortex, separate zones of cortex have been identified as having major roles in defensive behaviors, manipulation of objects, and hand-to-mouth grasping. Stimulation of the defensive zone, for example, elicited a squint and facial grimace and other defensive movements. In addition, a defensive zone where similar movements could be evoked was found in a portion of posterior parietal cortex (PPC) corresponding to the previously defined ventral intraparietal (VIP) area (8). Because VIP has interconnections with ventral premotor cortex (9–12), possibly including cortex where the defensive zone of frontal cortex is located, the VIP region and part of premotor cortex may be critical nodes in a network mediating defensive behaviors (2). Furthermore, because PPC, including the intraparietal region, contains a number of proposed subdivisions with differing connection patterns with motor and premotor cortex (9–15), other parts of PPC might interact with parts of frontal cortex as nodes in systems for various biologically relevant complex behaviors.

We evaluated this general hypothesis by stimulating PPC in prosimian galagos. These prosimian primates have many of the functional subdivisions of neocortex of anthropoid primates (monkeys, apes and humans), but galagos have generally simpler brains (16). Thus, galagos have the major divisions of premotor cortex of anthropoid primates (17), while having a less expansive PPC of possibly simpler organization. Although the sensorimotor systems of all animals must contain networks for brain functions such as protecting the body from attack (1, 2), networks for other ethologically relevant motor behaviors seen in monkeys, such as reaching and grasping, might be basic only to primates, because early primates occupied a fine-branch, arboreal niche where neural mechanisms for mediating reaching and grasping in bringing food to the mouth could be very important (18, 19). The present research was based on the premise that neurons involved in such distinct behaviors may be located in spatially separate parts of PPC, and that subdivisions of cortex related to basic primate behaviors might be more readily revealed in the less expansive PPC of galagos and other prosimian primates. Because our microstimulation results did reveal different functional zones in PPC of galagos, we studied the connections of these zones. As expected, each zone had a different pattern of connections with premotor and motor areas of the frontal lobe, as well as with other areas of parietal and occipital cortex.

Materials and Methods

The organization of PPC was studied in seven adult galagos (Otolemur garnetti) by using intracortical microstimulation. The connections of PPC were investigated in four of these galagos and in two additional galagos. All procedures were approved by the Vanderbilt University Animal Care and Use Committee, and followed National Institutes of Health guidelines.

Microstimulation. Galagos were initially anesthetized with 2% isoflurane; a portion of the skull was removed over PPC, and the dura was retracted. For the microstimulation periods, the anesthetic was a mixture of ketamine (~30 mg/kg) and xylazine (~5 mg/kg). It was delivered intravenously with an infusion pump at a rate that maintained a surgical level of anesthesia. The region of PPC was electrically stimulated at an array of sites by using a low-impedance (1 MΩ) tungsten microelectrode inserted perpendicular to the cortical surface to a depth of 1.5–1.8 mm, which was optimal for eliciting movements. Electrode penetrations were placed in a grid, with 0.5–1 mm between penetrations. Penetration sites were marked on a high-resolution digital photograph of the exposed cortex. Sites along the medial and lateral banks of the intraparietal sulcus were stimulated by advancing the electrode down these banks to a depth of up to 5 mm. Stimuli consisted of 500-ms trains of 0.2-ms biphasic pulses of current at 300 Hz. These stimulus trains match those previously used to obtain complex movements from motor, premotor, and PPC of macaque monkeys, and they approximate the time scale of normal reaching and defensive movements (1, 4, 5). Current pulses ranged from 20 μA to 300 μA, starting at lower values at each site and increasing or decreasing the current level to determine a threshold. Microlesions were placed at certain stimulation sites to mark functional borders and other sites of

Abbreviations: PPC, posterior parietal cortex; VIP, ventral intraparietal; FEF, frontal eye field.

*To whom correspondence should be addressed. E-mail: jon.h.kaas@vanderbilt.edu.

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interest by passing a direct current of 10 μA during the time the electrode was withdrawn from the cortex.

**Injection of Tracers.** Cortex was exposed under aseptic conditions, key sites were identified by microstimulation, and injections of anatomical tracers were placed within functional subdivisions of PPC. Tracers included biotinylated dextran amine, cholera toxin subunit B, and several fluorescent dyes, as described elsewhere (20). After injections, the dura was replaced, the skull was sealed with dental acrylic, and the skin was sutured. Prophylactic antibiotics and analgesics were given. After 5–7 days, each animal was reanesthetized, and further observations were made by using microstimulation.

**Histology and Data Analysis.** At the end of microstimulation sessions, each animal was given a lethal dose of sodium pentobarbital and, when areflexive, perfused with 0.9% PBS followed by 2% paraformaldehyde fixative and then fixative with 10% sucrose as described elsewhere (20). Cortex was separated from the rest of the brain, flattened, and cut parallel to the surface into 40-μm sections. A set of 1 in 4 of these sections was stained for myelinated fibers (21), and another set was processed for cytochrome oxidase (22). As needed, an additional set was mounted for fluorescent tracers, and other sets were processed for biotinylated dextran amine or cholera toxin subunit B (see ref. 20). Labeled neurons were localized in drawings of brain sections, including architectonic boundaries, with a microscope coupled to an X-Y plotter. Physiological results were superimposed, by using microlesions as landmarks.

**Analysis of Evoked Movements.** The patterns of movements evoked by microstimulation were recorded on videotape and classified by type (hand-to-mouth, reaching, and defensive) by two or three observers during the experiment and later by observing a digital video recording. Movement sequences were drawn from frames of the video record.

**Results**

The physiological and anatomical results support the broad conclusion that PPC of galagos contains two main sectors. A caudal sector was densely connected with non-primary visual cortex and was not excitable with microstimulation. A rostral sector differed in having sites throughout where stimulation evoked complex movement patterns. Sites for different classes of movements were clustered to reveal functionally distinct zones. Rostral PPC received visual inputs from caudal PPC, and inputs from somatosensory fields, motor fields, premotor fields, and frontal eye fields (FEFs). The focus of this report is on the microstimulation results.

**Evoked Movement Patterns.** Long, 500-ms trains of pulses effectively evoked complex movement patterns at sites throughout the rostral half of PPC. Shorter trains of 60 ms (17) occasionally evoked a muscle twitch from stimulation sites in PPC, but no complex movements. The type of movement evoked from PPC depended on the stimulation site. Stimulation usually evoked movements of the contralateral body parts, but some bilateral movements were observed. At no site was movement evoked exclusively on the ipsilateral body side. Most movements were initiated within 33–100 ms after stimulation onset. Face, eye blink, and ear movements were generally less delayed (33–67 ms) than forelimb movements (67–100 ms). Movements generally continued during the duration of the stimulus train, but forelimb movements sometimes stopped during stimulation.

One type of movement was classified as aggressive in that it seemed to resemble a threat (Fig. 1A). Possibly, this movement could also be considered a confrontation. The constellation of movements involved a blink followed by contractions of muscles around the eye causing a squint. The mouth opened, and the upper lip was withdrawn to expose the upper teeth. The pinna was rotated backward and downward and sometimes flattened against the sides of head. Defensive arm movements were those that involved retraction of the contralateral arm, elbow and wrist flexion, and rotation of the forearm with the hand turning outward as if to fend off an attack (Fig. 1B). The defensive face movements involved an eye blink and a face grimace combined with backward and downward ear movements. Sometimes these defensive face movements were associated with defensive forelimb movements described above. The hand-to-mouth movements included shaping of the hand into a grip posture followed by movement of the hand toward the mouth while the wrist and forearm rotated to orient the glabrous hand toward the mouth.

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**Fig. 1.** Classes of movements evoked by microstimulation of different zones of rostral PPC of galagos. Each segment of drawing depicts first and last phases of a type of complex movement traced from video recordings. (A) The aggressive face pattern included a backward folding of the ear, a squint of the eye, and an opening of the mouth to expose the teeth. (B) The forelimb defensive movement included a positioning of the forelimb near the face with the palm out as if to ward off a blow. (C) The hand-to-mouth movement brought the grasping hand to or near the mouth. (D) The reaching movement was a reach in front of the galago with a grasp.
neurons in secondary somatosensory fields, middle motor cortex, and dorsal premotor cortex (green arrows). Injections in the rostral half of PPC labeled different areas. Injections into the ventral part of this rostral zone labeled neurons in some non-primary visual areas, secondary somatosensory fields, ventral motor and premotor cortex, and the FEF (yellow arrows). Injections into the dorsal part of this rostral zone labeled neurons in secondary somatosensory fields, middle motor cortex, and dorsal premotor cortex (green arrows).

(Fig. 1C). Sometimes these movements were accompanied by an opening of the mouth. The reaching movements involved extending the arm in front of the animal, often ending with a dorsal flexion of the wrist and a closing of the digits in a grasping movement (Fig. 1D).

Hindlimb movements were evoked from a medial cluster of sites (Fig. 2A) and generally consisted of bilateral hip, knee, and ankle flexion, with the hip movement being the strongest. Hindlimb movements usually occurred in conjunction with defensive forelimb movements, although hindlimb movements alone were evoked from a few of the most medial sites. Eye movements consisted of horizontal saccades into contralateral visual hemifield. The electrical stimulation period was sometimes followed by a period of spontaneous eye movements.

The Organization of PPC. The rostral half of PPC was divided into separate zones for defensive, aggressive, reaching, hand-to-mouth, hindlimb, and eye movements (Fig. 2A). The rostral portion of PPC containing these zones for complex movements extended to 1–2 mm of primary somatosensory cortex (S1 or area 3b), but did not include the band of somatosensory cortex caudal to S1 that may correspond to area 1 (Fig. 2B). Injections of tracers in zones for complex movements labeled neurons in primary motor cortex (M1), dorsal and ventral premotor areas, and the FEF. Ventral PPC was more extensively connected to ventral parts of M1 (devoted to face movements), ventral premotor cortex (devoted to face and forelimb movements), and the FEF (Fig. 2C). The ventral part of the complex movement region also received inputs from the more caudal visual portion of PPC. Both dorsal and ventral portions of the movement region received inputs from secondary somatosensory areas of the lateral sulcus (21). The connections of the caudal portion of PPC were quite different, as injections labeled neurons in V2, V3, DM, DL, MT, and other visual areas (see refs. 24 and 25 for a description of these visual areas in galagos). The proportion of neurons labeled in these visual areas, and the areas involved, varied with the location of the injection site in the caudal half of PPC.

Microstimulation results across the seven extensively studied hemispheres varied somewhat, while reflecting the general pattern of Fig. 2A. Conclusions are based on the results of stimulating 907 sites in and around the intraparietal sulcus, and 125 sites anterior to the intraparietal sulcus. Movements were evoked from the cortex just 1–2 mm caudal to S1 (defined architectonically) to the mid-position of the intraparietal sulcus in all cases. Detailed results from two galagos are shown here.

Fig. 3 shows results from a typical galago (04-04). The rostral part of the explored region was stimulated with short 60-ms trains to locate motor areas for reference. Simple movements were evoked from sites in motor and premotor cortex and from somatosensory cortex by these trains. However, such short stimulus trains did not evoke movements from the PPC. In addition, the most caudal sites in PPC failed to evoke any movement even with long stimulus trains. These sites corresponded to the portion of PPC with direct inputs from visual areas. A broad band of PPC, between somatosensory cortex and the unresponsive PPC zone, contained all of the sites where complex movements were evoked with long trains of electrical pulses. This movement region could be further divided into at least six regions devoted to different classes of movements. Just ventral to the rostral half of the intraparietal sulcus, and including part of the cortex of the lower bank of the sulcus, sites evoked defensive movements of the face when stimulated. Most often, stimulation of these sites caused the contralateral eye to close entirely, the ipsilateral eye to partially close, the face to contract into a grimace, and the ear to fold back. Across all cases, stimulation of 328 of 359 sites in this “defensive face” region produced repeatable, short latency defensive-like movements of the face at current levels in the 100- to 150-μA range, but sometimes as low as 20 μA and occasionally at the maximum tested level of 300 μA. The nearly complete pattern of both eyes closing combined with the grimace was evoked at 267 sites, of which 249 sites also included ear retraction. Ten sites included shoulder retraction, 24 sites included only eye blink, and 7 sites included only ear retraction. In all studied galagos, the defensive face region extended dorsally to include part of the lower bank of the intraparietal sulcus, and, in one galago (04-04), the region also

Fig. 2. A summary of PPC organization in galagos based on present results. (A) A photograph of part of the exposed left cerebral hemisphere of a galago with functionally distinct movement zones outlined and colored. See Evoked Movement Patterns for descriptions of the movement patterns. The regions of anterior somatosensory cortex and motor cortex are indicated, and the intraparietal (IPS), lateral (LS), anterior frontal (FSA), and the posterior frontal (Fsp) sulci are marked. (B) A dorsolateral view of galago brain with the area of interest shown in A in a box. Primary (V1), secondary (V2), and middle temporal (MT) visual areas, somatosensory areas S1 and 3a, primary motor cortex (M1), premotor cortex (PM), and FEF are labeled for reference. The superior temporal sulcus is apparent just ventral to MT. (C) A summary of patterns of projections revealed by injections of retrogradely transported tracers into PPC. Injections of tracers into the caudal half of PPC labeled neurons in visual areas, including MT, DL (the dorsolateral area), the dorsomedial area, V3, and V2. White arrows indicate projections from these fields. Injections in the rostral PPC labeled different areas. Injections into the ventral part of this rostral zone labeled neurons in some non-primary visual areas, secondary somatosensory fields, ventral motor and premotor cortex, and the FEF (yellow arrows). Injections into the dorsal part of this rostral zone labeled neurons in secondary somatosensory fields, middle motor cortex, and dorsal premotor cortex (green arrows).
included the deep portions of the upper bank of the intraparietal sulcus (Fig. 3B). The region of eye closure and face movements extended along the most ventral part of the movement zone. At the caudal border of the defensive face zone of galago 04-04, several sites evoked saccadic eye movements, defining the eye movement zone (Fig. 3A). Such sites were found in three of seven cases (51 sites). Current levels of 200–300 μA were necessary to produce eye movements.

An aggressive face zone was just rostral to the defensive face zone (Fig. 3A). Although the movement complex evoked from this region included eye closures, and sometimes ear retraction, it also included opening of the mouth and retraction of the upper lip to expose the teeth. Across individuals, this movement complex was evoked at 56 sites. In 30 of these sites, arm retraction or (less often) arm extension occurred. Movements involving the lower limbs were usually bilateral.

The cortex dorsal to the intraparietal sulcus, including some of the cortex of the upper bank of the sulcus, was devoted to arm movements. Usually, as in galago 04-04 (Fig. 3), the most caudal part of this dorsal movement region was involved in reaching movements. Across individuals, extension of the arm with grasping was evoked at 32 sites in the reaching zone, and grasping with bringing the hand toward or to the mouth was evoked at 115 sites. In one animal (04-07, Fig. 4), the reach zone was not found, although the grasping hand-to-mouth movements were evoked from the caudal sites.

A zone for defensive forelimb movements was located in cortex just rostral to the forelimb zone for reaching. Across individuals, defensive movements, such as retracting and lifting the forelimb into the space beside the head, were evoked at 217 sites. Defensive forelimb movements involved ear retraction at 31 sites, and ear retraction alone was evoked at 12 sites. Movements were evoked in the 70- to 250-μA range. Sites for reaching and sites for defense movements were somewhat mixed in some cases (Fig. 3).

Just medial to the defensive forelimb zone, defensive forelimb movements were associated with hindlimb movements (24 sites). The hindlimb movements were usually bilateral.

Microstimulation results shown for a second case (Fig. 4) are quite comparable with those in Fig. 3. Simple movements were not evoked from PPC with short trains of stimulation, and long trains evoked complex movements from the rostral, but not caudal, portion of PPC. Sites for hand-to-mouth movements were mixed with sites for defensive forelimb movements in cortex dorsal to the intraparietal sulcus. An eye movement region lateral to this sulcus was not found. Thus, results varied somewhat across cases, but the same general pattern was always apparent. Movements involving the lower limbs were evoked only from the most dorsal sites, forelimb movements were related to more ventral sites, and face movements involved the most ventral sites. The sites related to different types of hand and forelimb movements were differentially distributed in the rostrocaudal dimension, whereas some mixing of sites occurred.

Discussion

The results indicate that complex movements can be evoked by microstimulation from the rostral half of the PPC of galagos. The
types of movements vary by location within this responsive region in a manner that suggests the existence of a number of zones that are specialized for roles in different behaviors. The complex movements seem to be components of ethologically meaningful behavioral patterns such as feeding and defense, and they resemble those previously evoked from posterior parietal (5–7) and frontal (1–4) cortex of macaques.

Graziano and coworkers (1, 2) used long (500-ms) trains of electrical pulses when stimulating motor and premotor cortex of awake macaque monkeys to reveal zones for different types of complex, behaviorally relevant movements: a manipulation zone largely coextensive with the hand representation of traditional primary motor cortex (M1), a defensive zone for protective movements that included part of dorsal premotor cortex, and a hand-to-mouth grasping zone that overlapped part of ventral premotor cortex. These results suggested to the investigators that motor and premotor areas could be considered a single large field with regional specializations for ethologically relevant behaviors. Our previous studies of motor cortex organization in galagos suggested an organization into primary motor and premotor areas (17) much like the current views of motor cortex organization in macaques (26–29). Using longer stimulation trains in the present cases, we found sites in motor and premotor cortex that evoked complex movements, although we have not yet studied this cortex extensively enough to make meaningful comparison with macaques. However, we found zones for complex behaviors in the PPC, including zones that seemed to be involved in defensive, aggressive, hand-to-mouth, and reaching behaviors. Thus, behaviors that were at least superficially similar to those evoked from macaques were revealed in the present study, but more categories of behavior were evoked in galagos, and the behaviors were evoked from PPC rather than frontal cortex.

That similar patterns of complex movements can be evoked from PPC of galagos and frontal cortex of macaques is not surprising. The complex movements elicited by the long trains of electrical pulses likely activate a node in a complex circuit that includes neurons in a number of cortical and subcortical structures. A number of functional divisions of PPC have been proposed for macaques (13, 30), and some of these subregions are densely, but variously interconnected with motor and premotor fields (9–15). Stimulating motor and premotor fields would activate parts of PPC, and vice versa. Thus, stimulating in either brain region could reveal similar complex behaviors. Indeed, there is already evidence from macaques that the movement patterns elicited from PPC of galagos likely resemble those previously evoked from posterior parietal cortex (PPC) of macaques, defensive movements were evoked that were similar to that elicited from dorsal premotor cortex (6). VIP and the premotor defensive region seem to be interconnected parts of a circuit for such complex behaviors (2).

Although little is known about the connections of PPC in galagos, our anatomical results indicate that the region involved in complex movements is interconnected with frontal motor and premotor areas and that functionally different zones in PPC connect in different patterns to these frontal motor areas. Thus, the movement patterns elicited from PPC of galagos likely depend, at least in part, on interconnections with motor and premotor fields. In our study, complex movements were elicited from a few sites in galago motor cortex. However, further study is needed to see whether sites in motor and premotor cortex of galagos reliably elicit comparable patterns of complex movements. Alternatively, it may be that the proper circuits are most readily accessed by electrical stimulation in PPC. In either case, the important conclusion to be drawn from the results in macaques and galagos is that circuits for ethologically relevant behaviors can be activated by electrically stimulating given locations in the cortex of both primates. Given the distant relationship of prosimian galagos and anthropoid macaques, with perhaps 70 millions years of separation (16), it is likely that all primates have brain circuits for such ethologically relevant behaviors. It remains to be seen when these circuits emerge in development and whether they depend on or are modified by experience.

An apparent difference between galagos and macaques is that more zones of functionally distinct movement patterns were observed in galagos than in PPC of macaques. Although present results seem to suggest that PPC in galagos is orga-
nized in a different way than in macaques, it is probably premature to come to this conclusion. A number of alternative schemes have been presented for the organization of this cortex in macaques (see ref. 30 for review), and the results of long trains of microstimulation have been reported only for the VIP area, where defensive movements were elicited (5). The defensive face region of galagos may correspond to the medial intraparietal area (MIP) of macaques (34, 35), spe-


cialized for grasping and object manipulation. These and other similarities raise the possibility of a number of homologous areas in PPC of prosimian and anthropoid primates. However, visual inputs to PPC in galagos seem to be largely limited to cortex caudal to the complex movement zones, whereas movement-related zones of VIP, LIP, and PRR of macaques all get inputs from subdivisions of visual cortex (8, 10, 13, 14). Thus, the large posterior parietal region of macaques is also likely to differ in organization in some respects from the much smaller posterior parietal region of galagos. Further comparative studies may reveal more details about the organization of PPC in these and other primates and suggest when and how functional subdivisions of PPC in humans evolved.

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