

Research Note

Cortico-cortical connections of two electrophysiologically identified arm representations in the mesial agranular frontal cortex

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Summary. Neuronal tracers (diamidino yellow or wheat germ agglutinin conjugated with horseradish peroxidase) were injected in the arm representations of area 6 α (mesial surface, area F3), in the arm representation of area 6 β (mesial surface) as well as in the eye field of area 6 β (dorso-medial surface). The results showed that the arm representation of area F3 receives topographically organized afferents from motor and premotor areas (areas F1, F2, F4 and F5). A further connection was found with that part of cingulate cortex that sends projections to the spinal cord. In contrast, the arm representation of area 6 β receives afferents chiefly from area F5, the prefrontal cortex and that part of cingulate sulcus which has few, if any, connections with the spinal cord. No connections were found with the precentral motor cortex (area F1). The area 6 β eye field receives afferents mostly from the frontal eye field. Further connections are with the prefrontal cortex and cingulate gyrus. It is suggested that the so called “low level” motor functions of supplementary motor area are due to the activity of area F3, whereas the so called “high level” motor functions depend upon an independent area located in area 6 β .

Key words: Mesial agranular frontal cortex – SMA – Cortico-cortical connections – Macaque monkey

Introduction

A series of findings, accumulated in recent years, suggest that the mesial part of the agranular frontal cortex,

classically referred to as the “supplementary motor area” (SMA), is a heterogeneous region formed by two or more distinct functional areas. Firstly, histochemical studies have shown that the caudal part of the mesial agranular frontal cortex (area F3) differs from its rostral part (Matelli et al. 1985). This finding is in good agreement with the classical subdivision of the agranular frontal cortex into two sectors called respectively area 6 α and 6 β (Vogt and Vogt 1919). Secondly, the rostral and the caudal parts of the mesial agranular frontal cortex appear to have different thalamic inputs. The caudal part receives afferents mostly from the nucleus ventralis lateralis pars oralis (VLo), whereas the rostral part is chiefly connected with area X (Schell and Strick 1984; Miyata and Sasaki 1984; Wiesendanger and Wiesendanger 1985). Thirdly, recent microstimulation experiments demonstrated that a complete somatotopic representation exists in the caudal part of the mesial agranular frontal cortex (Mitz and Wise 1987; Matelli et al., in preparation) and that this representation is coextensive with area F3 (Matelli et al., in preparation). Fourthly, neurons responding to passive somatosensory stimuli are abundant in the caudal part of the so called “SMA”, but are absent in its rostral part (Hummelsheim et al. 1988). Finally, single neuron recordings demonstrated that in the cortex rostral to F3, cytoarchitectonically coincident with area 6 β , there is an arm representation distinct from that of area F3 and separated from it by a mouth-face field (Rizzolatti 1989; Rizzolatti et al. 1990).

The aim of the present experiment was to study the connections of the arm fields of areas F3 and 6 β with the precentral motor cortex (area F1) and the premotor areas (areas F2, F4 and F5) by injecting fluorescent dyes and wheat germ agglutinin conjugated with horseradish peroxidase (WGA-HRP). In addition, since electrical

stimulation and single neuron recordings, performed to identify different somatotopic representations, showed that arm and eyes are represented in different parts of area 6a β , tracers were injected also in these fields. The results showed that the arm representations of areas F3 and 6a β have markedly different connections. The former link essentially motor areas, whereas the latter concern area 6a β with inferior area 6 and the prefrontal cortex, but not with the precentral motor cortex.

Methods

The present study is based on observations from 3 macaque monkeys (*Macaca fascicularis*). Under Ketamine anesthesia (15 mg/kg/i.m. supplemented every 30 min) and using procedures previously described (Gentilucci et al. 1988) a chamber was implanted for intracortical microstimulation (ICMS). Tungsten microelectrodes

(impedance 0.5–1.5 M Ω measured at 1 KHz frequency) were used for delivering a train of cathodal pulses generated by a constant current stimulator. Train duration: 50 ms, pulse duration: 0.2 ms, frequency: 330 Hz, current intensity: 3 to 40 μ A. The current strength was controlled on an oscilloscope by measuring the voltage drop across a 10 K Ω resistor in series with the stimulating electrode. The same electrodes were also used for unit recordings. The right mesial agranular frontal cortex was explored systematically in the 3 monkeys. Vertical transdural electrode penetrations were made in a 1 \times 1 mm grid extending 5 mm lateral to the midline over a 20 mm rostrocaudal extent of superior frontal gyrus.

The caudal part of the mesial agranular frontal cortex (area F3) was found to be electrically excitable (see for similar observations Mcpherson et al. 1982) and somatotopically organized (Mitz and Wise 1987). Arm movements were elicited from it with relatively low electrical currents (10–20 μ A). In contrast, the rostral mesial surface of area 6 (area 6a β) was mostly unexcitable (see also Mcpherson et al. 1982). However, movements could be also evoked from area 6a β by increasing the stimulus train duration (100 ms) and/or by applying them at the end of an active movement or when the animal

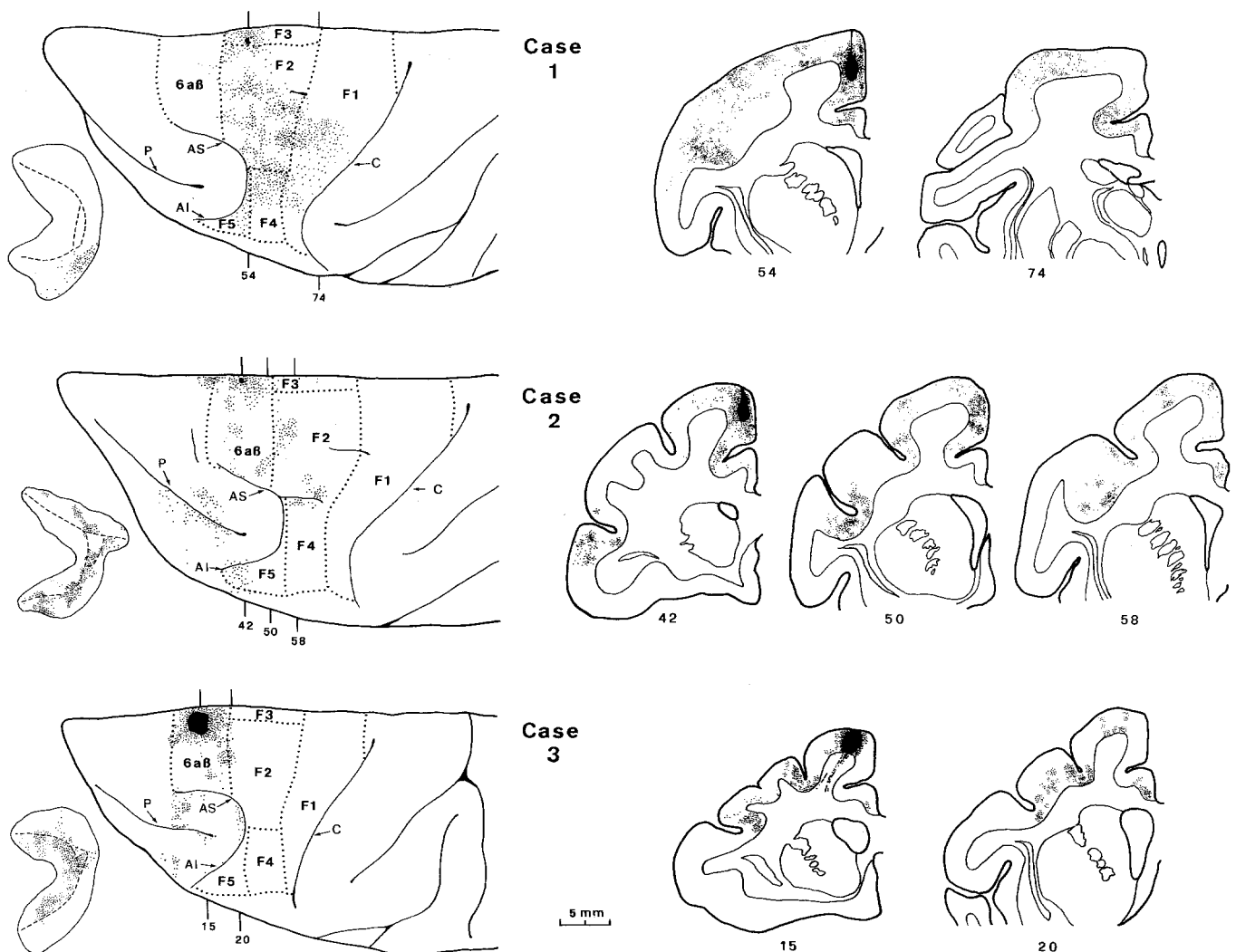


Fig. 1. Distribution of retrogradely labeled neurons following tracer injections in the mesial and dorso-medial agranular frontal cortex. Right side of the figure: selected coronal sections showing the distribution of labeled neurons in the three cases injected. The injection site is drawn in black. Each dot corresponds to a single marked neuron. Left side of the figure: reconstructions of the distribution of the retrogradely labeled cells on the dorsolateral

brain surface and within the arcuate sulcus (opened view). The density of dots represents the density of labeled neurons. Interrupted lines indicate the sulcal fundi. Dotted lines indicate borders between cyto- and chemo-architectonic areas. Abbreviations: AI = inferior arcuate sulcus; AS = superior arcuate sulcus; C = central sulcus; P = principal sulcus

relaxed immediately after it. Most of the evoked movements following mesial 6a β stimulation concerned the upper limb. Eye movements were also observed (see Schlag and Schlag-Rey 1987), but the eye field did not coincide with the arm field. In all monkeys it was located on the dorso-medial surface of area 6a β .

Once the systematic mapping of the right hemisphere had been completed, selected penetrations (ICMS, unit recordings) were made in the left hemisphere in order to identify, on the basis of the electrophysiological properties described above, the cortical fields chosen for the injection of the neuronal tracers. In this way we were able to inject physiologically identified fields and, simultaneously, to avoid the danger of possible damage consequent to extensive physiological mapping. All injections were made with a 1 μ l Hamilton microsyringe. Each monkey received a single injection of the tracer.

In the first animal (case 1) diamidino yellow (DY, 2%, 0.2 μ l) was injected in the arm representation of area F3. The injection was made in the mesial cortex at a depth of 3.5 mm. In the second monkey (case 2) 0.2 μ l of the same tracer were injected in the arm representation of area 6a β . The injection was made in the mesial cortex at a depth of 3 mm. In the third monkey (case 3) WGA-HRP (4%, 0.08 μ l) was delivered in the eye field of area 6a β . The injection was made in the cortical convexity at a depth of 1.5 mm. Following a survival time of 12 days, the monkeys injected with DY were deeply anesthetized with Nembutal and perfused transcardially with 4% paraformaldehyde in 0.1 M phosphate buffer pH 7.4. The third monkey (survival period of 48h) was anesthetized and perfused using the fixation procedures described in Mesulam (1982). The brains were cut in serial coronal sections at 60 μ m thickness using a freezing microtome. The first section of each 5 was mounted and air dried for the fluorescent observation (case 1 and case 2) or reacted for HRP histochemistry (case 3) using TMB as chromogen (Mesulam 1982). The location of each labeled neuron in cortical sections was charted on a X-Y plotter which is fed from two linear potentiometers coupled to the movements of the microscope stage. In order to identify the borders of chemo- and cytoarchitectonic areas of the agranular frontal cortex, the second and the fifth section of each 5 were reacted for cytochrome-oxidase histochemistry (Matelli et al. 1985) and stained by the Nissl method respectively.

Results

Figures 1 and 2 show the injection sites and the corresponding labeled areas in the frontal and cingulate cortex of the three monkeys studied in the present experiment. In *case 1* the injection site was in the arm region of area F3. On the dorsal surface (Fig. 1) a large number of labeled neurons were found in superior area 6 (area F2). Other sources of afferents were the rostral part of the arm representation of area F1 (area 4) and the arm representation of area F4 (inferior area 6). Less dense clusters of labeled cells were found in the posterior bank of arcuate sulcus (area F5). Finally, scattered labeled cells were also observed in the lateral part of area 6a β . On the mesial surface (Fig. 2) a dense population of labeled cells was present in the whole arm field of area F3. A few marked cells were also found rostrally in the ventral part of mesial area 6a β . In cingulate cortex, labeled neurons were located mostly in the ventral bank of the cingulate sulcus. This large projection originates chiefly from the caudal part of agranular cingulate area (area 24). A few labeled neurons were observed in the rostralmost part of the granular cingulate area (area 23).

In *case 2* the injection was in the arm field of mesial area 6a β . On the dorsal convexity (Fig. 1) the main

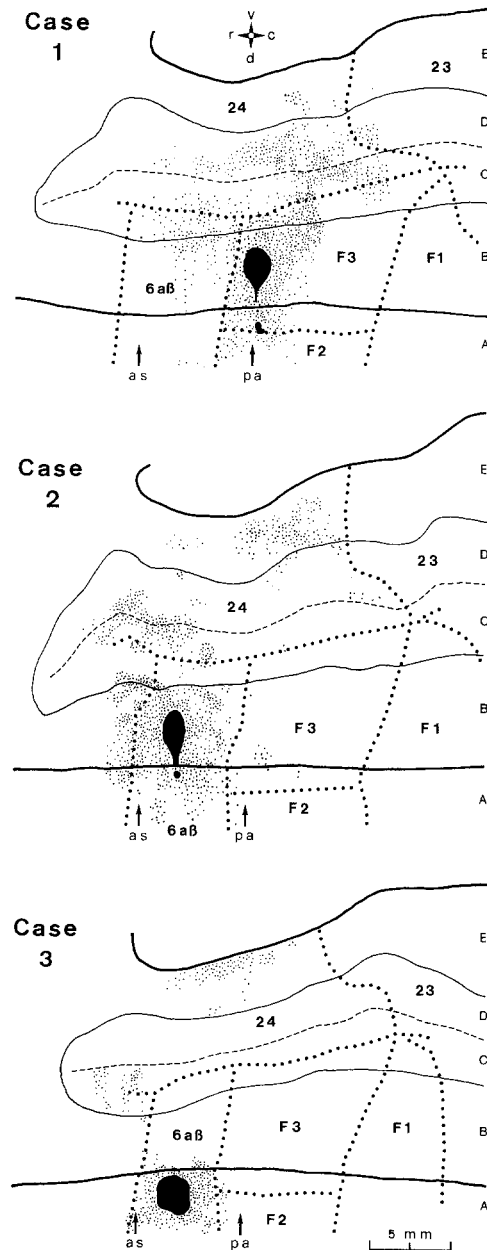


Fig. 2. Reconstructions of the distribution of the retrogradely labeled neurons on an unfolded view of the dorsomesial part of the frontal lobe. These maps are displayed using the conventions presented in Hutchins et al. (1988). A = dorsomesial cortical surface; B = mesial cortical surface; C = dorsal bank of the cingulate sulcus; D = Ventral bank of the cingulate sulcus; E = cingulate gyrus. Arrows indicate the anterior (as) and the posterior (pa) limits of the superior arcuate sulcus. The density of dots represents the density of marked neurons. All other conventions as in Fig. 1

source of afferents was in the posterior bank of the inferior arcuate sulcus (area F5). Scattered clusters of cells were present in areas F2, F4 and lateral 6a β . Note the absence of marked cells in area F1. A substantial number of labeled neurons were observed in the prefrontal cortex. The labeling extended dorsally and ventrally along the principal sulcus. On the mesial cortical surface (Fig. 2) labeled neurons were found in area 6a β . They were almost completely lacking in area F3. Marked cells

were also observed in two separate regions of agranular cingulate cortex. One was located along the fundus of cingulate sulcus, the other in the dorsal two thirds of the cingulate gyrus. Note that the rostral labeling had a location more anterior than that of case 1.

In *case 3* the injection was in the eye field of area 6a β (Schlag and Schlag-Rey 1987). Except for a small labeling in rostral area F2 and around the injection site, no labeled cells were found in the whole extension of motor and premotor areas (Fig. 1 and Fig. 2). The main source of afferents was in the anterior bank of the arcuate sulcus. Other marked cells were found in prefrontal areas, mainly in the dorsal part of the arcuate gyrus. In cingulate areas the labeled neurons were confined to two small regions of the agranular cingulate area, one in the dorsal bank of the cingulate sulcus, close to its rostral end, the other in the cingulate gyrus just above the corpus callosum.

Discussion

Two major findings emerge from the present study. a) A different pattern of cortico-cortical connections characterizes the two arm representations in the mesial agranular frontal cortex. b) The mesial and dorso-medial parts of area 6a β are linked with different cortical areas. Mesial 6a β is connected with areas which control arm movements, whereas dorso-medial area 6a β is mostly connected with areas which control eye movements.

The caudal arm representation is part of a somatotopically organized motor area which is electrically excitable with intracortical microstimulation (Mcpherson et al. 1982; Mitz and Wise 1987; Matelli et al., in preparation). Its cortical connections are chiefly with other motor areas. These connections appear to be topographically organized. In particular, the caudal arm field is connected with the arm representation of area F1 (area 4), with the arm representation of inferior area 6 (Gentilucci et al. 1988) and with superior area 6. Finally, as far as the connections with cingulate cortex are concerned, the caudal field receives afferents from that sector of area 24 which has heavy projections to the cervical spinal cord (Hutchins et al. 1988), and whose stimulation evokes arm movements (Matelli et al., in preparation).

Classically, the supplementary motor area (SMA) has been defined as a somatotopically organized motor area lying rostral to the primary motor cortex, on the mesial cortical surface (Penfield and Welch 1951; Woolsey et al. 1952). This definition fully applies to the motor representation located in area F3. This area sends direct connections to the spinal cord (e.g. Murray and Coulter 1981; Mcpherson et al. 1982; Hutchins et al. 1988), is electrically excitable and contains a complete representation of body movements (Mitz and Wise 1987). The organization of its cortico-cortical connections, as revealed by the present experiments, as well as the above mentioned anatomo-functional properties, suggests that F3 plays a role in the motor control at a rather late stage.

Unlike the arm field of area F3, the arm field of area 6a β is not connected with the precentral motor cortex

and is only loosely connected with areas F2 and F3. Its "motor" connections are mostly with area F5 which, cytoarchitectonically, is a disgranular cortex (Von Bonin and Bailey 1947) and, functionally, codes goal-directed motor acts (Rizzolatti et al. 1988). In addition, the arm field of area 6a β receives higher order information from the prefrontal cortex. These findings suggest for this area a role in motor control more related to movement preparation than to movement execution. Congruent with this point of view is the lack of descending pathways from area 6a β to the spinal cord (Kuypers 1981; Keizer and Kuypers 1989) and the presence of neurons which are related to reaching-grasping movements, but fire in large advance of the actual movement (Rizzolatti 1989; Rizzolatti et al. 1990). These neurons, which in some aspects resemble the set-related cells described by Wise and his coworkers (Weinrich and Wise 1982; Wise and Mauritz 1985), are influenced by the distance of the objects from the monkey and, frequently, show rather complex excitation-inhibition patterns depending upon whether the animal can or cannot reach objects (Rizzolatti et al. 1990).

An interesting issue that has been debated in the recent years concerns the so called "high level" motor functions of the SMA (see Wiesendanger 1986). These functions have been proposed mostly on the basis of two series of observations made in man: the occurrence of slow potentials in correspondence to the mesial frontal areas preceding primary motor cortex activation and movement onset (Deecke and Kornhuber 1978), and the observation of a blood-flow increase corresponding to the same cortical location during movement planning in the absence of overt movement (Roland et al. 1980). Although neurons which may mediate high level control functions have been reported in the mesial agranular frontal cortex of the monkey (e.g. Tanji and Kurata 1985; Tanji et al. 1987; Mann et al. 1988), a large number of monkey experiments "seem to indicate that the SMA exerts motor control functions at a relatively low level" (Wiesendanger 1986, p. 43). In order to reconcile this discrepancy it has been suggested that the SMA has two different functions, one related to the execution of movements ("low level" control function) and one to the preparation of them ("high level" control function) and that the caudal SMA is more involved in "lower" controls and the rostral SMA more in "higher" controls (Wiesendanger 1986). The data presented in this paper confirm this conceptual dichotomy. However they suggest also that the two different functions of the so called "SMA" are not due to the activity of the same area, but rather that F3 is the area responsible for the "low level" motor function of the mesial frontal cortex, whilst area 6a β is responsible for its "high level" motor function.

A last point worth discussing is the different pattern of cortico-cortical connections of the mesial and dorso-medial parts of area 6a β . The mesial part is connected with inferior area 6 and, although at limited extent, with the premotor areas where arm is represented. In contrast, the main connection of the dorso-medial part is with areas that control eye movements. This suggests that area 6a β is formed by two independent areas. Although

tempting, this conclusion, however, is only provisional. Before accepting it, more data are necessary on the thalamo-cortical input and especially on the detailed cytoarchitectonic organization of area 6a β .

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References

- Bonin G von, Bailey P (1947) The neocortex of *Macaca mulatta*. University of Illinois Press, Urbana
- Brinkman C, Porter R (1979) Supplementary motor area in the monkey: activity of neurons during performance of a learned motor task. *J Neurophysiol* 42:681-709
- Deecke L, Kornhuber HH (1978) An electrical sign of participation of the mesial 'supplementary' motor cortex in human voluntary finger movement. *Brain Res* 159:473-476
- Gentilucci M, Fogassi L, Luppino G, Matelli M, Camarda R, Rizzolatti G (1988) Functional organization of inferior area 6 in the macaque monkey. I. Somatotopy and the control of proximal movements. *Exp Brain Res* 71:475-490
- Hummelsheim H, Bianchetti M, Wiesendanger M, Wiesendanger R (1988) Sensory inputs to the agranular motor fields: a comparison between precentral, supplementary-motor and pre-motor areas in the monkey. *Exp Brain Res* 69:289-298
- Hutchins KD, Martino AM, Strick PL (1988) Corticospinal projections from the medial wall of the hemisphere. *Exp Brain Res* 71:667-672
- Keizer K, Kuypers HGJM (1989) Distribution of corticospinal neurons with collaterals to the lower brain stem reticular formation in monkey (*Macaca fascicularis*). *Exp Brain Res* 74:311-318
- Kuypers HGJM (1981) Anatomy of the descending pathways. In: Brooks VB (ed) *Handbook of physiology*, Vol II. Motor control, Part 1. American Physiological Society, Bethesda MD, pp 597-666
- Mpherson JM, Marangoz C, Miles TS, Wiesendanger M (1982) Microstimulation of the supplementary motor area (SMA) in the awake monkey. *Exp Brain Res* 45:410-416
- Mann SE, Thau R, Schiller PH (1988) Conditional task-related responses in monkey dorsomedial frontal cortex. *Exp Brain Res* 69:460-468
- Matelli M, Luppino G, Rizzolatti G (1985) Patterns of cytochrome oxidase activity in the frontal agranular cortex of macaque monkey. *Behav Brain Res* 18:125-137
- Mesulam MM (1982) Principles of horseradish peroxidase neurohistochemistry and their applications for tracing neural pathways. In: Mesulam MM (ed) *Tracing neural connections with horseradish peroxidase*. Wiley, Chichester, pp 1-152
- Mitz AR, Wise SP (1987) The somatotopic organization of the supplementary motor area: intracortical microstimulation mapping. *J Neurosci* 7:1010-1021
- Miyata M, Sasaki K (1984) Horseradish peroxidase studies on thalamic and striatal connections of the mesial part of area 6 in the monkey. *Neurosci Lett* 49:127-133
- Murray E, Coulter JD (1981) Organization of corticospinal neurons in the monkey. *J Comp Neurol* 195:339-365
- Penfield W, Welch K (1951) The supplementary motor area of the cerebral cortex. *Arch Neurol Psychiat* 66:289-317
- Rizzolatti G (1989) Functional properties of a mesial premotor area (area F6) in the macaque monkey. *Proc. XXXI Intern Congress Physiological Sciences*, S 5045
- Rizzolatti G, Camarda R, Fogassi M, Gentilucci M, Luppino G, Matelli M (1988) Functional organization of inferior area 6 in the macaque monkey. II. Area F5 and the control of distal movements. *Exp Brain Res* 71:491-507
- Rizzolatti G, Gentilucci M, Camarda R, Gallese V, Luppino G, Matelli M, Fogassi L (1990) Neurons related to reaching-grasping arm movements in the rostral part of area 6 (area 6a β). *Exp Brain Res* (press)
- Roland PE, Larsen NA, Shinhoj E (1980) Supplementary motor area and other cortical areas in organization of voluntary movements in man. *J Neurophysiol* 43:118-136
- Schlag J, Schlag-Rey M (1987) Evidence for a supplementary eye field. *J Neurophysiol* 57:179-200
- Schell GR, Strick PL (1984) The origin of thalamic inputs to the arcuate premotor and supplementary motor areas. *J Neurosci* 4:539-560
- Tanji J, Kurata K (1985) Contrasting neuronal activity in supplementary and precentral motor cortex of monkeys. I. Responses to instructions determining motor responses to forthcoming signals of different modalities. *J Neurophysiol* 53:129-152
- Tanji J, Okano K, Sato KC (1987) Relation of neurons in the nonprimary motor cortex to bilateral hand movement. *Nature* 327:618-620
- Vogt O, Vogt C (1919) *Ergebnisse unserer Hirnforschung*. *J Psychol Neurol (Leipzig)* 25:277-462
- Weinrich M, Wise SP (1982) The premotor cortex of the monkey. *J Neurosci* 2:1329-1344
- Wiesendanger M (1986) Recent developments in studies of the supplementary motor area of primates. *Rev Physiol* 103:1-59
- Wiesendanger R, Wiesendanger M (1985) The thalamic connections with medial area 6 (supplementary motor cortex) in the monkey (*Macaca fascicularis*). *Exp Brain Res* 59:91-104
- Wiesendanger M, Hummelsheim H, Bianchetti M, Chen DF, Hyland B, Maier V, Wiesendanger R (1987) Input and output organization of the supplementary motor area: discussion. In: *Ciba Foundation Symposium 132. Motor areas of the cerebral cortex*, pp 53-62
- Wise SP, Mauritz KH (1985) Set-related neuronal activity in the premotor cortex of rhesus monkeys: effects of changes in motor set. *Proc R Soc L B* 223:331-354
- Woolsey CN, Settlage PH, Meyer DR, Sencer W, Pinto Hamuy T, Travis AM (1952) Patterns of localization in precentral and "supplementary" motor areas and their relation to the concept of a premotor area. *Res Publ Assoc Nerv Ment Dis* 30:238-264