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## Deficits in manipulative behaviors induced by local injections of muscimol in the first somatosensory cortex of the conscious monkey

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Injection of muscimol (GABA agonist) in the finger region of area 2 of the alert monkey led to the striking yet reversible loss of finger coordination, thus disabling the monkey from picking up a small piece of food from a small hole or a funnel. Symptoms varied depending on sites of injection within the finger region. Neither weakness of hand or finger movements nor deficit in hand reach was observed.

The first somatosensory cortex (SI) of the monkey is composed of areas 3, 1 and 2. The differences in receptive field properties of cells in areas 3, 1 and 2 have been well documented: receptive fields become larger and more complex posteriorly from area 3b to area 2<sup>6,9</sup>. Unlike area 3b, where neurons are organized basically in a somatotopic manner<sup>8</sup>, areas 1 and 2 are composed of clusters of those neurons which frequently have different receptive fields or submodalities<sup>9,11–13</sup> but are related to a specific behavior of active touch to 3-dimensional objects<sup>5,7,10,13</sup>. Area 2 sends a substantial amount of efferent axons to the motor cortex<sup>14,18,21</sup>, and thus it is possible that each of these neuronal clusters in area 2 provides the motor cortex with organized sensory information in the control of specific hand behaviors. One way to test this hypothesis is to see if a localized inactivation of neurons in area 2 impairs a specific hand behavior of the monkey. To achieve this task we injected a small amount of muscimol, a potent agonist of  $\gamma$ -aminobutyric acid (GABA)<sup>1</sup>, at various sites in the finger region of the alert monkey SI. GABA has been presumed to be an inhibitory neurotransmitter in the cerebral cortex<sup>16</sup>; iontophoretic injection of GABA suppresses spike activities of virtually every cortical neuron<sup>15</sup>. Therefore, local inactivation of neurons by

muscimol would mimic a localized surgical lesion. This technique has been proven to be very useful for studying the functions of the superior colliculus<sup>4</sup> in the alert monkey.

Under general anesthesia using Nembutal, devices for head restraint and a cylinder for single cell recording and chemical injection were implanted on the SI of a female, adult Japanese monkey (*Macaca fuscata*). We at first recorded single cell activities in the SI cortex using a glass-coated platinum–iridium (P1-Ir) microelectrode and determined their characteristics. After removing this electrode we inserted a pipette for injection and recording under the binocular scope through the same hole on the dura resulting from the preceding electrode penetration. The injection–recording pipette was made of glass, connected to a Hamilton syringe (5  $\mu$ l) by a polyethylene tube and contained a thin, glass-insulated tungsten microelectrode. The pipette was held by a device that allowed us to move the tungsten electrode independently up or down through the glass pipette while keeping the space in the pipette air-tight. A saline solution of muscimol was sucked into the pipette just before each injection while the rest of the space was filled with liquid paraffin. The tungsten electrode was then lowered so as to fit the glass pipette; here

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the tip of the electrode protruded out of the orifice of the glass pipette (diameter: 30–50  $\mu\text{m}$ ) by 100–200  $\mu\text{m}$ . An injection of muscimol was made after the monkey's behavior was tested by a set of control experiments. Just before the injection, the tungsten electrode was raised by 50–100  $\mu\text{m}$  independently of the glass pipette to make space between them. Through this space muscimol was pressure-injected by small steps. The concentration of muscimol solution was either 1 or 5  $\mu\text{g}/\mu\text{l}$ , and a total of 1.5  $\mu\text{l}$  was injected for each experiment. After 30–60 min the injection site was marked by passing currents (10  $\mu\text{A}$ , 30 s) through the tungsten electrode.

Changes in the monkey's behavior following the injection were examined using various tasks that required the monkey to manipulate her hand and fingers in precise or complex ways to pick up small pieces of apple or food pellets from small holes on a wooden block or various containers. In most cases the monkey was allowed to use only one hand: before each trial the monkey's hands were gently held by the investigator; upon the release of one of her hands the monkey extended her freed hand to retrieve the food. These tests were done before the injection and at various intervals after the injection. They were done also without the monkey's vision by blindfolding her eyes; in this case the release of the monkey's hand signalled to her that the wooden block or one of the containers with reward food was available. The performance on these tests was recorded on videotapes using two cameras (30 frames/s) and was subjected to frame-by-frame analyses. The whole sequence of the monkey's performance was timed by an internal timer the output of which was displayed on each frame. Injection and recording sites were reconstructed histologically using the Klüver–Barrera method.

A total of 18 injections of muscimol was made in the hand–finger region of the SI cortex of the two hemispheres of a monkey. Changes in tactile behavior were detected following 15 injections in SI (4 out of 4 in area 3b; 2 out of 2 in area 1; 8 out of 11 in area 2; 1 out of 1 in area 3a). The behavioral changes varied depending on the injection sites and necessitated various behavioral tasks.

In this communication we illustrate one example in which muscimol (7.5  $\mu\text{g}$  in 5  $\mu\text{g}/\mu\text{l}$  saline) was injected in the anterior part of area 2 of the left SI. Six

neurons were isolated along the penetration using a Pt-Ir microelectrode (Fig. 1A). They were activated by passive displacement of the tissue around the nail (units 1, 2, 3, 5, 6) or flexion (units 4, 6) of the 3rd to 5th fingers of the contralateral (right) hand, the 4th finger being the most effective (Fig. 1B). None of them responded to gentle rubbing of these fingers using a paint brush. However, by far the most intense discharges occurred in units 4–6 when the monkey attempted to pick up a small cut piece of apple from a small hole (diameter, 20 mm; depth, 20 mm) on the wooden block (Fig. 1C). As illustrated in Fig. 1C, right, and Fig. 2A, PRE, the 2nd (index) finger was stretched and inserted into the hole to search for the apple piece, while the 3rd to 5th fingers were just flexed with the dorsal surface of their distal segments kept on the surface of the wooden block.

A local injection of muscimol disrupted this behavior (Fig. 2A, POST). The injection site is shown by an arrow in Fig. 1A. Neural activities recorded before the injection using the tungsten microelectrode in the injection pipette were virtually the same as shown in Fig. 1B. The 3rd to 5th fingers of the right (contralateral to the injection) hand lost the stable, flexed configuration, and repeated clumsy flexion and extension. Due to this lack of stability and perhaps partly because of the simultaneous flexion and extension of the 2nd finger, it took a much longer time for the monkey to pick up the apple piece. While in this case the monkey was blindfolded, the finger coordination was only slightly improved when the monkey could see her own hand. Such disruption of the monkey's performance had good correlation with the characteristics of neurons close to the injection site. That is, the functional loss of a group of neurons, which discharged most vigorously when the 3rd to 5th fingers assumed a stable, fixed position while the 1st (thumb) and 2nd fingers picked up a piece of apple, led to the disruption of the stable position of the 3rd to 5th fingers and consequently disturbed the normal performance of the 1st and 2nd fingers.

The lack of finger coordination was more clearly revealed when the monkey was required to pick up a piece of apple from a funnel (Fig. 2B), although the monkey was not blindfolded. The left (ipsilateral to the injection) hand showed a series of movements normally seen in this task (Fig. 2B, IPSI). While the monkey reached out her left hand, its shape was al-

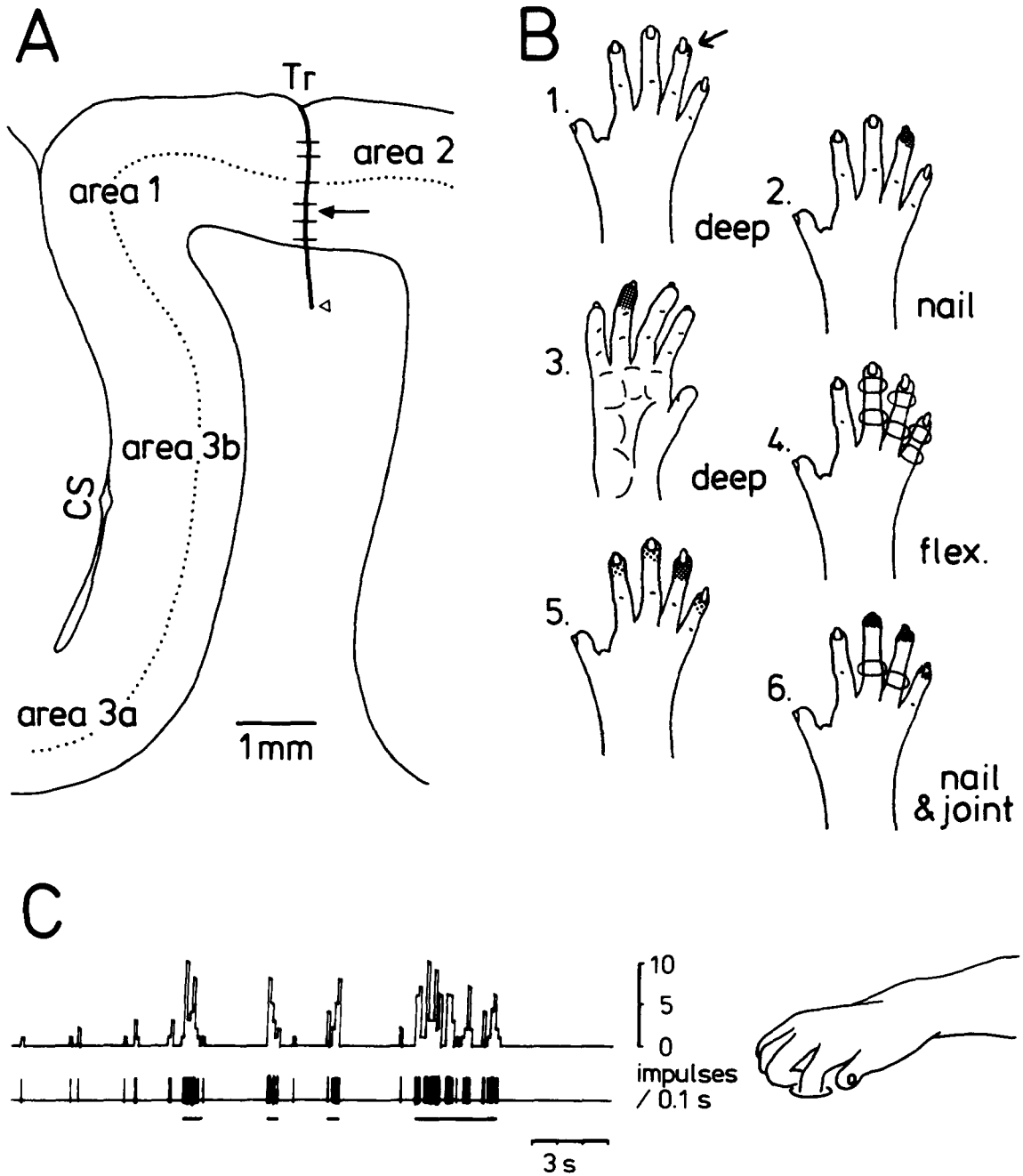


Fig. 1. Site of muscimol injection and cell characteristics. A: parasagittal section of the left SI, finger region. Muscimol was injected in the anterior part of area 2 (arrow). Tr indicates the track of the recording electrode and the pipette for injection. CS, central sulcus. B: response properties of 6 single neurons recorded along the penetration through the injection site; recordings were made before the injection. Receptive fields are shown as shaded areas. Effective sites of joint manipulation are indicated by circles over the joints; effective direction denoted as flex (flexion) C: vigorous discharges of neuron 4 in B while the monkey attempted to pick up a small piece of apple from a small hole (diameter, 20 mm, depth, 20 mm) with her right hand. instantaneous discharge rate (above) and spike density (below); underlined period indicates the monkey's attempt to pick up the apple piece.

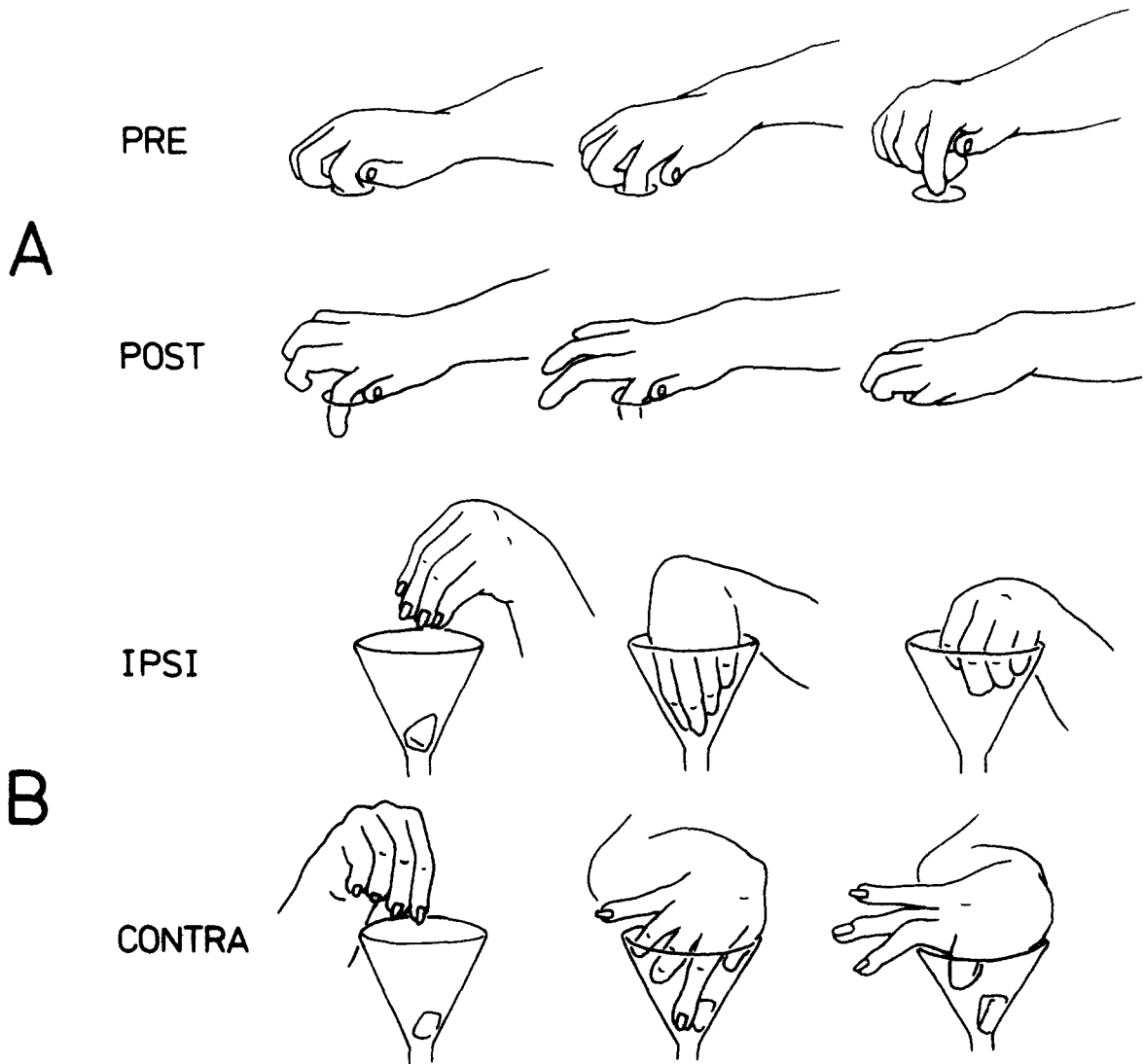


Fig 2. Disruption of finger coordination following the muscimol injection in area 2. A: inability to pick up an apple piece from the small hole. Sequential from left to right. Fifty-seven min after the injection (POST) the 3rd to 5th fingers repeated clumsy flexion and extension, which contrasted with the stable position before the injection (PRE). B: sequence of finger coordination (left to right) to pick up an apple piece from a funnel (2 h and 10 min after the injection). IPSI shows the normal pattern by the left hand; CONTRA shows severely disorganized pattern by the right hand. The monkey was blindfolded in A but not blindfolded in B.

ready adapted to that of the funnel, all fingers being put close together and slightly flexed. This funnel-shape of the left hand allowed the 2nd and 3rd fingers to reach the bottom of the funnel and scoop up the piece of apple. It took only 0.2–2.3 s (mean, 1.2 s; 10 trials) for the left hand to touch the funnel, pick up the apple piece and withdraw. Before the injection the right hand showed the same pattern and skill of movements. After the injection (Fig. 2B, CONTRA), however, the right hand was severely disorganized. As soon as the fingers touched the inner wall

of the funnel, the 4th and 5th fingers were extended and sometimes the 3rd finger as well protruded outside the funnel repeating flexion and extension in vain. The 2nd or 3rd finger, therefore, barely reached the bottom of the funnel, and attempts by the 1st and 2nd fingers to pick up the apple piece were successful in only 4 out of 14 trials. It took 1.2–3.2 s (mean, 2.3 s) on the 4 trials for the monkey to pick up an apple piece.

We did not notice any abnormality when the monkey reached with her right hand to a distant object,

grabbed objects of various shapes, ate a half-cut orange while manipulating it with both hands, groomed the arm of one of the investigators, or walked on the floor. The above symptoms, as in other experiments, lasted more than 5 h but completely disappeared on the 1st examination of the next day (about 20 h after the injection).

Disorganization of fingers was one of the most common symptoms after a muscimol injection in area 2 of the hand region. Symptoms varied, however, with different injections even in the small area 2 hand region: injections separated by 2 mm usually produced readily distinguishable symptoms. This suggests that the areas inactivated by the muscimol injections were fairly small. One injection in area 2 (1st and 2nd finger region) made it difficult for the monkey to pick up a piece of apple from one of the small holes on the wooden block by opposing 1st and 2nd fingers if she was blindfolded.

These symptoms contrasted with those after injections in area 3b or area 1. The initial sign after the injections in area 3b or 1 was usually the inability to detect a piece of food placed in the hole whereas no deficit was obvious in the shape of the hand or the pattern of finger movements (unlike area 2 injections). For example, when muscimol was injected in the 2nd finger region of area 3b, the monkey groped in the small hole with her 2nd finger but frequently ignored the piece of food; if a piece of food was in a small plastic cylinder, she repeatedly gripped its wall with the 1st and 2nd fingers and brought her hand to her mouth apparently assuming that the piece of food

was between her fingers.

In patients with lesions of the postcentral gyrus, Foerster<sup>3,19</sup> described motor disturbances in the contralateral limb. The patients could not perform the required movements of fingers, such as to button a shirt, to write letters or to pick up small items. It was especially difficult to use opposing thumb and index fingers, since other fingers also moved purposelessly. When requested to reproduce various finger positions, the patients gave undifferentiated quasiathetoid movements. Some investigators have also noted motor disturbances in the monkey following postcentral lesions, loss of ability to discriminate object shapes by active palpation<sup>2</sup>, slowness in the initiation and execution of movements<sup>20</sup>, loathness to move<sup>2,20</sup> and awkwardness in the posture<sup>2,17</sup>. These symptoms, however, have drawn less attention since then, and analysis of the disturbances has not been done satisfactorily.

The symptoms caused by muscimol injection in the present study may be compatible with some of these classical observations in monkeys and human patients with lesions of the postcentral gyrus. Our results confirm that the postcentral gyrus is crucial in the control of fine finger movements especially in the manipulation of objects. We demonstrated further that within the postcentral gyrus area 2 is most responsible for this task. It would provide the motor cortex with spatially organized information concerning contacting surfaces, shapes or other features of objects, or kinesthetic schemes of specific finger movements.

1 Andrews, P. R. and Johnston, G. A. R., GABA agonists and antagonists, *Biochem. Pharmacol.*, 28 (1979) 2697–2702.

2 Cole, J. and Glees, P., Effects of small lesions in sensory cortex in trained monkeys, *J. Neurophysiol.*, 17 (1953) 1–13.

3 Foerster, O., Symptomatologie der Erkrankungen des Gehirns, In O. Bumke and O. Foerster (Eds.), *Handbuch der Neurologie, Band 6, Allgemeine Neurologie IV*, Springer, Berlin, 1936, pp. 308–322.

4 Hikosaka, O. and Wurtz, R. H., Effects on eye movements of a GABA agonist and antagonist injected into monkey superior colliculus, *Brain Res.*, 272 (1983) 368–372.

5 Iwamura, Y. and Tanaka, M., Postcentral neurons in hand region of area 2: their possible role in the form discrimination of tactile objects, *Brain Res.*, 150 (1978) 662–666.

6 Iwamura, Y., Tanaka, M. and Hikosaka, O., Overlapping representation of fingers in the somatosensory cortex (area

2) of the conscious monkey, *Brain Res.*, 197 (1980) 516–520.

7 Iwamura, Y., Tanaka, M. and Hikosaka, O., Cortical neuronal mechanisms of tactile perception studied in the conscious monkey. In M. Sato and R. Norgren (Eds.), *Brain Mechanisms of Sensation, IIIrd International Symposium, Division of Brain Sciences, The Taniguchi Foundation*, J. Wiley, New York, 1981, pp. 61–70.

8 Iwamura, Y., Tanaka, M., Sakamoto, M. and Hikosaka, O., Functional subdivisions representing different finger regions in area 3 of the first somatosensory cortex of the conscious monkey, *Exp. Brain Res.*, 51 (1983) 315–326.

9 Iwamura, Y., Tanaka, M., Sakamoto, M. and Hikosaka, O., Converging patterns of finger representation and complex response properties of neurons in area 1 of the first somatosensory cortex of the conscious monkey, *Exp. Brain Res.*, 51 (1983) 327–337.

10 Iwamura, Y., Tanaka, M., Sakamoto, M. and Hikosaka,

- O , Functional surface integration submodality convergence and tactile feature detection in area 2 of the monkey somatosensory cortex, *Exp Brain Res* , Suppl , in press
- 11 Iwamura, Y., Tanaka, M , Sakamoto, M and Hikosaka, O , Comparison of the hand and finger representation in area 3b, 1 and 2 of the monkey somatosensory cortex. In M. Rowe and W D. Willis (Eds.), *Development, Organization and Processing in Somatosensory Pathways*, Alan R Liss, New York, in press
  - 12 Iwamura, Y , Tanaka, M , Sakamoto, M and Hikosaka, O , Diversity in receptive field properties of vertical neuronal clusters in the crown of the postcentral gyrus of the conscious monkey, in preparation.
  - 13 Iwamura, Y , Tanaka, M., Sakamoto, M. and Hikosaka, O., Neuronal clusters in the postcentral gyrus signaling active touch A receptive field study in the first somatosensory cortex of the conscious monkey, in preparation
  - 14 Jones, E. G , Coulter, J D. and Hendry, S., Intracortical connectivity of architectonic fields in the somatic sensory, motor and parietal cortex of monkeys, *J comp Neurol.*, 181 (1978) 291-348
  - 15 Krnjević, K and Phillis, J W . Ionophoretic studies of neurones in the mammalian cerebral cortex. *J Physiol (Lond )*, 165 (1963) 274-304
  - 16 Krnjević, K and Schwartz, S , The action of  $\gamma$ -aminobutyric acid on cortical neurones, *Exp Brain Res* , 3 (1967) 320-336
  - 17 Kruger, L and Porter, P , A behavioral study of functions of Rolandic cortex in the monkey, *J comp Neurol* , 109 (1958) 439-469
  - 18 Kunzle, H., Cortico-cortical efferents of primary motor and somatosensory regions of the cerebral cortex in *Macaca fascicularis*, *Neuroscience*, 3 (1978) 25-39
  - 19 Luria, A R , *Higher Cortical Functions in man*, Basic Books Institute Publishers, New York, 1966, pp. 189-207.
  - 20 Peele, T L , Acute and chronic parietal lobe ablations in monkeys, *J. Neurophysiol.*, 7 (1944) 269-286
  - 21 Vogt, B A and Pandya, D N , Cortico-cortical connections of somatic sensory cortex (areas 3, 1 and 2) in the rhesus monkey, *J comp Neurol* , 177 (1977) 179-192