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RESPONSES OF NEURONES IN MOTOR CORTEX AND IN AREA 3A TO CONTROLLED STRETCHES OF FORELIMB MUSCLES IN CEBUS MONKEYS

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SUMMARY

1. The experiments were designed to investigate the effects of longitudinal muscle displacements on neurones of the motor cortex of anaesthetized Cebus monkeys and thus test the hypothesis that signals from muscle spindles may modify motor cortical output. The effects of sinusoidal stretching of the extensor digitorum communis (EDC) at frequencies varying from 6 to 300 Hz and of step and rhomboidal stretches were studied in neurones of the motor cortex. For comparison, neurones of the primary receiving area for low-threshold muscle afferents, cortical area 3a, were also included in this study. Neurones of the motor cortex were subdivided into corticospinal (PT) neurones and non-corticospinal (non-PT) neurones.

2. Threshold stretch amplitudes were clearly higher for neurones of area 4 (PT and non-PT) than for 3a neurones. However, a conspicuous fall in threshold stretch amplitude was observed for all three neurone populations when the frequency of sinusoidal stretching was increased (highest frequency: 300 Hz). A small number of non-PT and PT neurones responded to vibration amplitudes of less than 100 μm and some of these low-threshold cells of area 4 also responded to rhomboidal stretches of 8 mm/sec ramp velocity and 80 μm plateau amplitude. Increasing the stretch amplitude to twice threshold nearly doubled the output magnitude in all three cell types. Neurones of area 3a and non-PT neurones of area 4 had similar latencies, and these were significantly shorter than the latencies of PT neurones tested with trains of high frequency vibration. Dynamic response patterns were observed in all three cell types, but most frequently in 3a neurones.

3. It is concluded that, in Cebus monkeys, signals from both primary

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and secondary muscle spindle endings from forelimb muscles reach the motor cortex. Under the present experimental conditions, the input from the primaries to the motor cortex was effective only if these spindle receptors were driven maximally by vibratory stimuli. The particularly low probability of stretch-evoked discharges of cortico-spinal neurones in the anaesthetized preparation may be explained by a low gain in transmission from input to output cells of the motor cortex.

INTRODUCTION

The present experiments were designed to further test the hypothesis that motor cortical output may be automatically adjusted when increased load impedes a movement. In such a servo-loop, first proposed by Phillips (1969), the error signals to the cortex may be provided by muscle spindles in response to increased load, i.e. to a 'misalignment' between intra- and extrafusal muscle fibres (Merton, 1953). Since the above hypothesis was formulated, several reports have been published (Melvill Jones & Watt, 1971; Marsden, Merton & Morton, 1972, 1973; Evarts, 1973; Conrad, Matsunami, Meyer-Lohmann, Wiesendanger & Brooks, 1974; Tatton & Lee, 1975) which strongly support the view of a modulation of motor output induced by external perturbations of movements *via* loops involving supraspinal structures. These important studies in man and non-anaesthetized monkeys did not, however, allow a detailed analysis of the components of the proposed servo-loop. It was apparent that acute experiments were needed to elucidate the receptors and pathways involved. In a first attempt (Phillips, Powell & Wiesendanger, 1971), graded electrical stimulation of muscle and skin afferents of forelimb nerves was used to obtain information about the type of afferents and the timing of cortical responses. In the baboon, low-threshold muscle afferents were shown to project with secure and rapid transmission to area 3a at the depth of the central sulcus. Single brief muscle stretches at amplitudes of 70 μm and trains of vibration (400 Hz, 50 μm) were also capable of exciting 3a neurones. It was therefore concluded that signals from primary muscle spindle endings had a potent excitatory effect on cells of area 3a which lie adjacent to the motor cortex. Neurones of area 3a failed to respond antidromically to strong stimulation of the dorsolateral funiculus of the cervical cord (Phillips *et al.* 1971; Heath, 1973) and therefore do not represent the efferent limb of a transcortical servo-loop. No evidence was found that 'pure' group I volleys were capable of modulating motor cortical cells (Wiesendanger, 1973). Stronger stimuli, viz. above group II thresholds, were necessary to excite neurones of the motor cortex at latencies appropriate for long loop muscle responses to muscle stretch.

This interpretation does not preclude the possibility that muscle spindle primaries may also be involved because a short train of about 1/2 maximum group I volleys (i.e. without group II contamination) may be too weak to excite motor cortical cells.

In the present experiments, stretch stimuli of controlled parameters were therefore applied in order to generate maximal driving of muscle spindle primaries. Using single step displacement, ramp stretches, and sinusoidal stretches at low and high frequencies it was possible to determine the stimulus features which effectively excite motor cortical cells. It was especially important to compare the effects on the output cells to the spinal cord ('PT-cells') with those on 'non-PT' cells and on 3a neurones (which may represent the first link in the intracortical pathway) under the same experimental conditions. The results are consistent with the previous assumption that muscle spindle secondaries are the most important receptors to generate motor cortical output. Evidence was obtained, however, which suggested that muscle spindle primaries contribute as well.

METHODS

Anaesthesia and surgery. Twelve young Cebus monkeys weighing 1·4–2·5 kg were used. Anaesthesia was initiated with I.M. injections of phencyclidine hydrochloride (2–4 mg/kg) or ketamine hydrochloride (20 mg/kg) and maintained during surgery with small doses of sodium thiamylal (5 mg, I.V.); inhalation anaesthesia was administered (60 % N_2O in O_2) during recording together with further injections of sodium thiamylal at long intervals of 2 hr or more. In three monkeys, which would have required more frequent administration of thiamylal as judged by the occurrence of spontaneous movements (the monkeys were not curarized and the ventral roots were left intact), a single dose of 50 mg α-chloralose was injected I.V. All monkeys were maintained in good condition as evidenced by the normal blood pressure (100–120 mmHg), rectal temperature (36–38° C) and the normal appearance of the pial blood vessels. The right forelimb was routinely denervated except for the deep radial nerve. The uppermost part of the cervical cord was exposed to allow for placement of a pair of stimulating electrodes (short insect pins, insulated except 1 mm from the tip) into the right dorsolateral funiculus. The cisterna cerebello-medullaris was opened to allow drainage of cerebro-spinal fluid and thus to reduce brain pulsations. The left hand area of the peri-Rolandic cortex was exposed and protected by a pool of mineral oil.

Stretching of forearm muscles. The tendons of the extensor digitorum communis (EDC) and sometimes also of one of the wrist extensors were prepared and rigidly connected with 25 gauge stainless-steel wire to the stretcher. The tendon sheaths were opened to allow movement of the muscle with minimal friction. The arm was fixed (the forearm in semi-pronation) with steel pins at the elbow and wrist joints preventing movements in these joints. A gated function generator (Wavetek 144) and additional operational amplifiers provided the input signals for the stretcher which was controlled by position servo. The stretcher unit included a Ling-Altec Shaker (Model 203), and a Schaewitz HR-50 linear variable differential transducer with a Schaewitz CAS-200R carrier amplifier system. The transducer with the amplifier system had a frequency response allowing measurements of low-level

displacements up to 500 Hz. The position signal was calibrated microscopically with a micrometer eye piece. The position signal was used for the position control system by comparing it in a summing junction with the input signal from a buffer amplifier. The output from the summing junction was fed into two lead-lag networks which tended to compensate for the poor frequency response of the shaker and increased the gain of the system. The output from the lead-lag networks was then fed into the power amplifier whose output was limited (8·4 or 18·9 W r.m.s. into 3 Ω) to protect the shaker from damage. The position control system was found to have an overall stiffness of 60 N/mm and to operate accurately down to the low micron range. The maximal distortion-free displacements (18·9 W) were 5 mm at 50 Hz or below, 2 mm at 100 Hz, 380 μm at 200 Hz, and 130 μm at 300 Hz. A tension transducer was also built into the system. Four strain gauges arranged in a bridge circuit were mounted on a metal ring in series with the tendon. The muscle was pre-stretched from a length corresponding to 20 g tension by 6 mm ensuring high sensitivity of muscle spindles to the experimental stretching.

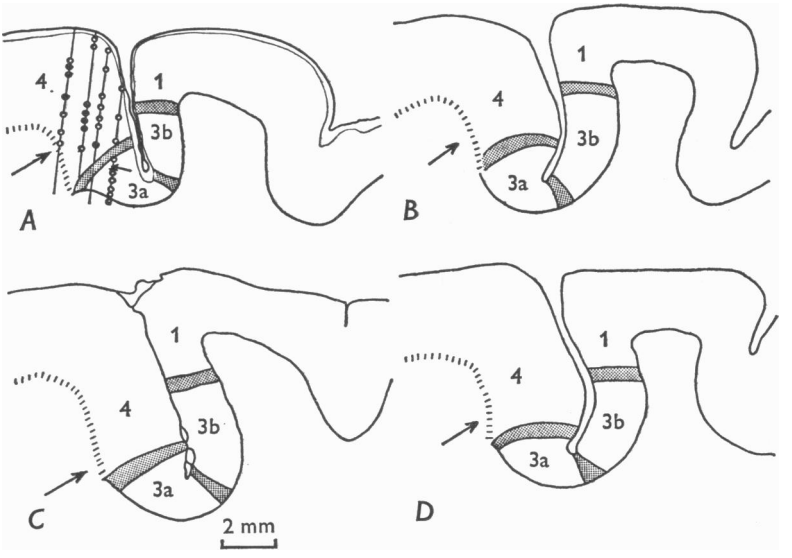
Stimulation and recording. E.m.g. responses elicited by motor cortical stimulation and by passive stretch were recorded by means of a pair of needle electrodes insulated except at the tip. A bipolar cuff electrode was used for stimulation of the intact deep radial nerve. Corticospinal neurones were identified by their ability to follow antidromic stimulation of corticospinal fibres at the C2–C3 level. Stable recordings conditions were achieved by the use of a floating micro-electrode which was similar to that described by Burns & Robson (1960) and which allowed prolonged recording even from smaller cells. The glass-capillaries were filled with 2 M-NaCl saturated with fast green dye for marking selected recording sites. Area 3a was first localized by searching for the field potentials evoked by deep radial nerve stimulation. Penetrations in the precentral cortex were usually within the low threshold area for eliciting twitches of the contralateral wrist and finger extensors by trains of anodal stimuli (12 pulses, 0·2 msec duration) applied to the cortical surface. These two areas and all points of micro-electrode penetrations were marked in each experiment on an enlarged photograph of the brain surface showing the detailed vascular pattern. The precentral track nearest the fissure and those deliberately aimed at area 3a were the most crucial ones for histological identification and were localized by leaving the capillaries *in situ* and/or by marking the deepest point of the penetration with fast green dye (Thomas & Wilson, 1965).

Data collection and analysis. Action potentials were amplified (filters were usually set at 100 Hz and 10 kHz) and fed into a window discriminator. Twenty responses were photographed from the oscilloscope as a dot-raster together with the signal from the position transducer and occasionally also with the signal from the tension transducer. In order to investigate marginal effects and also to recognize subtle modulations imposed by individual cycles of sinusoidal stretching, the activity of some selected units and the position signal were also recorded on analogue tape for analysis on a PDP-12 computer. Usually 100 stimuli were summed for post-stimulus time histograms (reference signal = gating signal for sinusoidal or step stretch) and cumulative distribution functions (slightly modified versions from STAP-12 library; Wyss & Handwerker, 1971).

The following experimental protocol was used: sinusoidal displacements at frequencies of 6, 12, 24, 48, 100, 200 and 300 Hz at threshold and $2T$ amplitudes; single step displacements of 50–100 msec duration at threshold and $2T$ amplitude; rhomboidal stretch with ramp velocity of 8 mm/sec and 80 μm plateau amplitude (for low-threshold cells, Text-fig. 8); electrical stimulation of peripheral nerves; electrical stimulation of dorsolateral funiculus, first with single pulses and, for antidromic vertification, with double pulses at minimal intervals (1–2 msec). The

complete investigation of each unit required about an hour. The minimal amplitudes for the various frequencies of sinusoidal stretching and for step displacements were measured from the filmed records; the latencies were measured from the responses obtained with $2T$ stimulation. The pattern of discharge (short burst, long burst, modulation with individual cycles, tonic discharge, inhibition) was also noted.

Histology. The animals were perfused under deep pentobarbitone anaesthesia with normal saline and then with 10% buffered formalin solutions. The right hemisphere was used for degeneration studies, small electrolytic lesions having been made in area 3a of the fore- and hind limb (to be reported separately). A block of pre- and post-central gyrus of the left cortex containing the recording tracks was cut serially in a parasagittal plane (frozen sections, 45 μm thick), and the sections stained with thionine. Most of the important electrode tracks and dye marks (see above) were found and correlated with the cytoarchitectonic features. The micro-electrodes penetrated the cortex in a slightly posterior–anterior direction. Thus, with penetrations just anterior to the central fissure the tracks traversed the motor cortex (Text-fig. 1 *A*).



Text-fig. 1. Boundaries of cortical areas 4, 3a, 3b, and 1 in four Cebus monkeys (*A*, *B*, *C*, *D*). Reconstruction from analysis of serial parasagittal sections (thionine, see Pl. 1). The boundaries are drawn as bands to illustrate the variability in interpretation of the dividing lines from section to section. Arrows indicate appearance of giant pyramidal cells in area 4. *A* is from an animal in which the buried cortex was tracked systematically, as illustrated, for one sagittal plane. The tracks were identified histologically and the depth reference was obtained from a fast green dye mark which is indicated by a small arrow in the most caudal track. Each cell encountered by the exploring micro-electrode was plotted in the track: non-PT cells by open circles, PT-cells (antidromically identified by stimulation of the contralateral dorsolateral funiculus) by filled circles. Note the lack of corticospinal neurones in area 3a and the abrupt appearance of corticospinal neurones in more rostral tracks.

RESULTS

Cytoarchitectonic and electrophysiological differentiation of area 3a and area 4

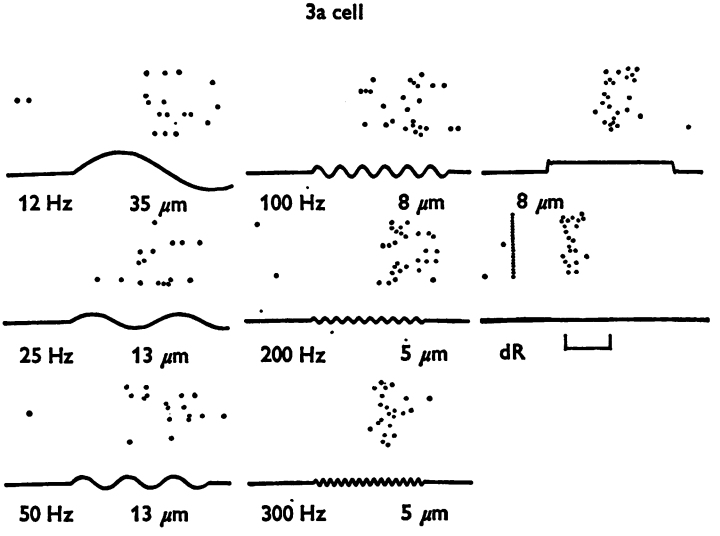
In the baboon, the boundaries of area 3a have been delimited and illustrated by Phillips *et al.* (1971). A good description of the cytoarchitectonic features of area 3a of the Cebus monkey was given by von Bonin (1938), but the boundaries were not illustrated. In the arm field, area 3a ('area postcentralis gigantopyramidalis' in von Bonin's nomenclature) is a narrow strip in the depth of the central sulcus. In the hind-limb field, however, area 3a is located more anteriorly and near the surface in front of the sulcus. As von Bonin's nomenclature implies, area 3a is characterized by some scattered large cells in the 5th layer. These large cells were often observed in the present histological material; they sometimes had a somewhat paler appearance than the gigantopyramidal cells of the motor cortex and were found in the most anterior portion of area 3a. The thickness of the cortex gradually increases from area 3b to area 4; the thinnest part of 3a was at the bottom of the sulcus and was regarded as the most caudal part of this area. In the border zone between area 3b and 3a the granular layer of area IV gradually thins out and disappears completely in the anterior part of area 3a. Characteristically, the sharp demarcation between the 6th fusiform layer and the white matter in the post-central cortex including area 3a disappears at the borderline between area 3a and area 4 (Pl. 1). It has to be pointed out, however, that an exact division between motor cortex and area 3a ('Mischzone' of Vogt & Vogt, 1919–20) is not possible, especially if based on one criterion only and on inspection of a single section. Text-fig. 1 illustrates the boundaries as plotted for the brains of four Cebus monkeys. It appears then that, for the forelimb, area 3a in Cebus monkeys is situated slightly more anterior than in the baboon (Phillips *et al.* 1971).

In two monkeys, a search for output cells to the spinal cord was carried out by systematic tracking, moving progressively from post-central to precentral cortex. Such an experiment is illustrated in Text-fig. 1*A*. Corticospinal cells were not found in area 3a (confirming results of Phillips *et al.* (1971) in baboon experiments), but were found rather abruptly anterior to the 3a–4 border zone. Thus, it appears that the cytoarchitectonic criteria which were used in the present study correlate well with functional criteria.

General features of responses to stretch in single units of area 3a

In order to ascertain that impulses generated by the stretch stimuli were reaching the cerebral cortex, one or more tracks per experiment were deliberately aimed at area 3a. The characteristic powerful and short

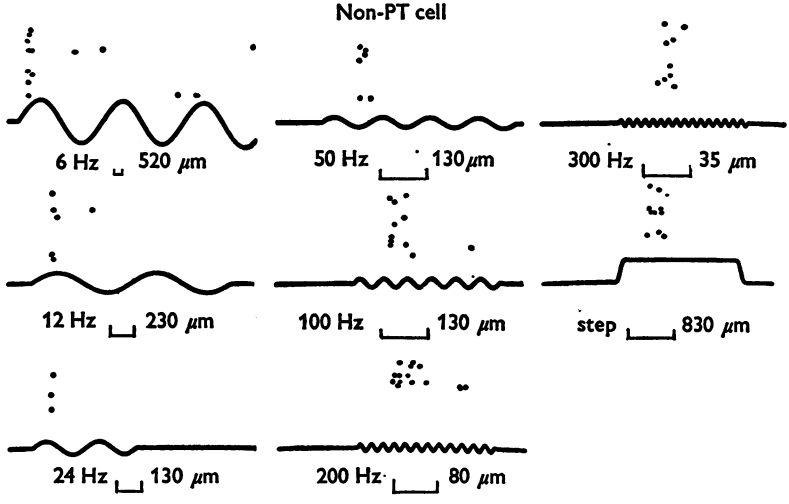
latency responses to low threshold stimulation of the muscle nerve were similar to those described in the baboon (Phillips *et al.* 1971): field potentials were elicited with single pulses at or just above threshold for eliciting EDC muscle twitches. The latencies of the field potentials ranged from 5·5 to 6·5 msec. The mean depth of recording was 6·1 mm (S.E. of mean ± 0·4). Sinusoidal stretches typically evoked short bursts of unitary discharges at low thresholds of displacements. The results of a full investigation are shown in an example in Text-fig. 2. There was a progressive



Text-fig. 2. Response pattern of a 3a neurone located in the depth of the post-central gyrus. Dot-raster of twenty consecutive responses to stretch stimuli which were just above threshold for the cortical cell at each frequency of sinusoidal stretching of the EDC muscle. Note progressive decrease of minimal amplitudes as the frequency was increased. Also illustrated are the responses to a minimal step displacement and to electrical stimulation of the deep radial nerve at twitch threshold (artifacts of single stimuli represented by vertical row of dots). Time calibration: 20 msec. In this and subsequent recordings stretch is indicated by an upward deflexion of the position trace.

decrease in threshold amplitudes as the frequency of sinusoidal stretching was increased up to 300 Hz. Discharges of 3a neurones typically occurred in bursts. Some intraburst-modulation imposed by the frequency of vibration was revealed in those neurones selected for statistical analysis of the spike trains; large and small peaks of activity occurred which could be correlated with the individual cycles of displacement. This activity was often followed by a silent period of up to 300 msec. These results

fully confirm those obtained in earlier experiments on baboons (Phillips *et al.* 1971), and the more detailed analysis of responses to controlled displacements of the forearm muscle will be used to compare these results with those from neurones of the motor cortex. The sample size of 3a neurones ($n = 21$) is relatively small because only a few such cells were deliberately recorded at the beginning of each experiment, the main goal being to record from cells of the motor cortex.

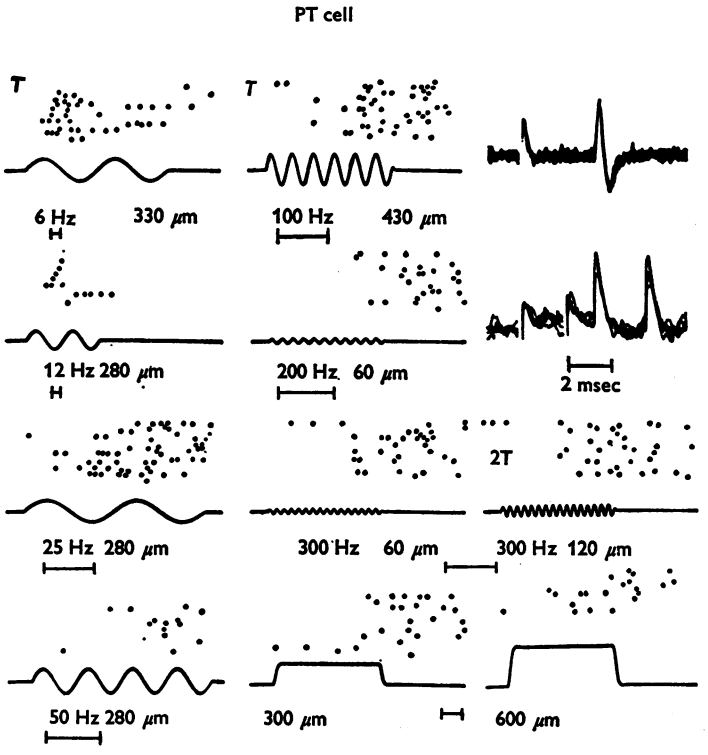


Text-fig. 3. Response pattern of a non-PT neurone of area 4 tested with the same experimental protocol as shown in Text-fig. 2. The amplitudes of displacements were kept just above threshold for the cortical cell. Time calibration: 20 msec.

General features of responses to stretch in single units of the motor cortex

The best site for stretch-evoked responses in the motor cortex was found to coincide with the area of lowest threshold for eliciting a twitch response of the contralateral hand and finger extensors to anodal cortical surface stimulation. The threshold intensity for an e.m.g. response varied between 0·4 and 1·1 mA. This area was found to be 2–4 mm more lateral than the 3a focus for afferents from the deep radial nerve. Stretch-evoked responses were observed in each experiment; often, however, many 'unsuccessful' tracks preceded successful ones. Once responding units were found, further tracks were made in a narrow zone around the first 'good' track. The impression in all experiments was that the input zone for the particular muscle afferents was limited to a narrow area of about 3 mm diameter near the central fissure confirming more systematic studies on cutaneous inputs to the motor cortex (Rosén & Asanuma, 1972). But

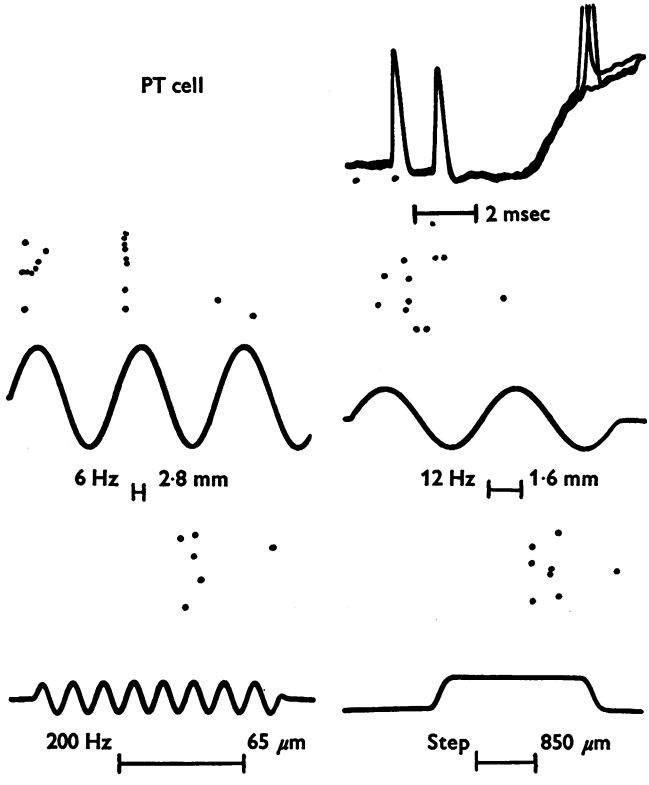
even within the input zone for muscle afferents, many cells were encountered which did not respond at all to any of the stretch stimuli. This was particularly true for corticospinal neurones. A total of eighty-three neurones in area 4 responded to muscle stretch; of these only twenty-one were corticospinal (PT) neurones, although a special effort was made to find stretch-evoked responses in these output cells of the motor cortex. The yield was better in the middle and superficial layers of the motor



Text-fig. 4. Discharge pattern of a slow-conducting corticospinal (PT) neurone. Antidromic responses (single and double shocks at minimal interval) to contralateral stimulation of the dorsolateral funiculus. The responses at threshold amplitudes of displacements consisted of long bursts ('tonic' response). Also illustrated is the response to 300 Hz vibration and step displacements at 2*T* amplitudes. Time calibrations for dot-rasters: 50 msec.

cortex from cells which were not identified as corticospinal. The approximate depth localization was as follows: for non-PT neurones seventeen cells in the superficial third of the cortex, thirty-one cells in the middle third, and fourteen cells in the deep third; for PT-neurones one cell in the superficial third, eight cells in the middle third, and twelve cells in the

deep third. Thus there was a concentration of responsive cells in the middle layers of the motor cortex, but responsive non-PT neurones were also found in a considerable number in the most superficial layers. Many neurones, especially in superficial and middle layers, were probably small because the extracellular spikes were small and they were easily 'lost' after small displacement of the micro-electrode. Stable recordings would have been impossible without use of a floating micro-electrode. A considerable number of such neurones which were responsive to stretch



Text-fig. 5. Discharge pattern of a fast-conducting corticospinal (PT) neurone. Antidromic and late orthodromic responses (five-times superimposed) elicited by a pair of electrical stimuli at 1 msec interval (indicated by two dots) applied to the dorsolateral funiculus. Illustrated are only the responses to selected frequencies to show partial driving of the cell with 6 Hz sinusoidal stretching at high displacement amplitudes and lack of driving when the frequency was increased to 12 Hz. Note the conspicuous fall in minimal displacement amplitude as high frequency vibration was used. The modulation of discharges with individual cycles of stretching and the short burst responses to vibration and step displacements were classified as 'dynamic' responses. Time calibrations for dot-rasters: 20 msec.

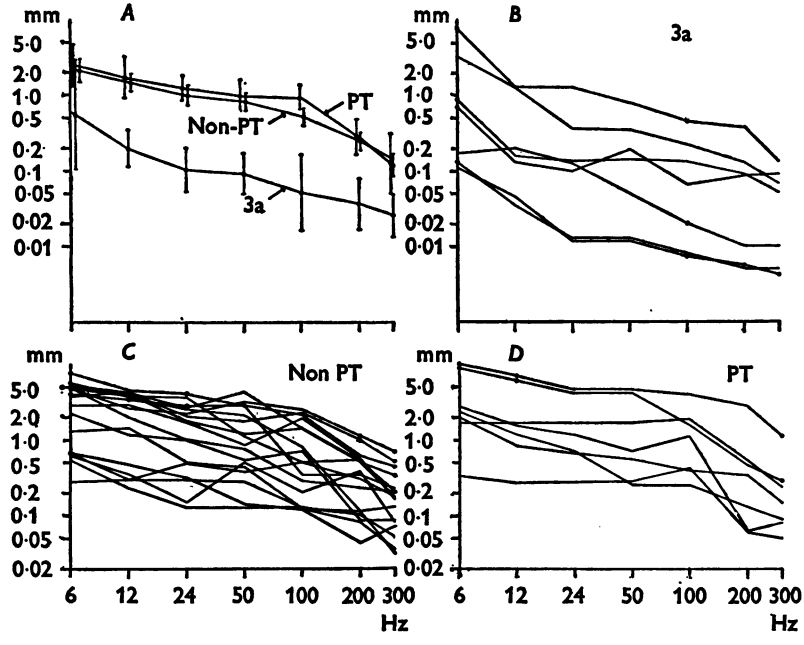
stimuli, but could only be tested in a rudimentary way, had therefore to be discarded from the following analysis. Text-figs. 3 and 4 illustrate results of testing a non-PT cell and a PT-cell of the motor cortex. The particular non-PT cell reacted with a short burst to all stimuli whereas the slow-conducting PT cell displayed tonic responses. However, short bursts or 'dynamic' responses of PT cells (see below for classification) were also encountered as illustrated in Text-fig. 5. E.m.g. responses to the various stretch stimuli were investigated in three experiments. Step displacements and the initial stretch of low-frequency sinusoidal displacements evoked, at a latency of 12 msec, a well synchronized e.m.g. response which was considered to be the monosynaptic stretch reflex. With trains of high frequency vibration, the response consisted of a burst occurring at a latency of 11 msec and with durations depending on the duration of vibration.

The deep radial nerve was cut at the end of three experiments. The field potential in the precentral gyrus evoked by a train of vibration was completely abolished in these control experiments.

Comparative analysis of response characteristics of the three cell types

Amplitude thresholds. In Text-fig. 6 the minimal effective stretch amplitudes are plotted for the various frequencies of sinusoidal stretching. For some units amplitude values for one or two frequencies were not available (dots on graphs of Text-fig. 7*B*, *C*, *D*) and were approximated by linear interpolation or extrapolation (taking into account also the measured amplitudes for the particular frequencies). These approximated values were included to calculate the means of the total sample in Text-fig. 6*A*. It appears from Text-fig. 6 that all three cell types responded at considerably lower amplitudes when the frequency of sinusoidal stretching was increased above 100 Hz and was lowest at 300 Hz (note logarithmic scale). At 300 Hz 3a neurones had a mean amplitude threshold of about 30 μm and the threshold values for some 3a neurones were in the same range as the threshold for primary muscle spindle endings (for instance 5 μm for the 3a neurone illustrated in Text-fig. 2). More surprising, however, was the fact that motor cortical cells were also more sensitive to high-frequency vibration than to low-frequency sinusoidal stretching, suggesting that signals from primary muscle spindle endings may also reach the motor cortex. This suggestion is furthermore supported by observations on eleven non-PT cells and on four PT cells with minimal thresholds below 100 μm. Wong, Kwan & Murphy (1974) recently reported that primary muscle spindle endings but not secondaries from the cat's forelimb responded to ramp stretches of 8 mm/sec velocity and of 80 μm amplitude. In the present material, such rhomboidal stretches were

utilized in sixteen selected cells which responded to low-amplitude vibration. Unequivocal responses were recorded in all three cell types (Text-fig. 7), viz. n three out of four 3a cells, in five out of eight non-PT cells, and in three out of four PT cells. Step displacements of the muscle evoked cortical responses at threshold amplitudes which were always higher than the threshold amplitudes of vibration: by a factor of 2·7 for 3a cells, by a factor of 9·5 for non-PT cells, and by a factor of 7·6 for PT cells. This clearly indicates that temporal summation played an important role.

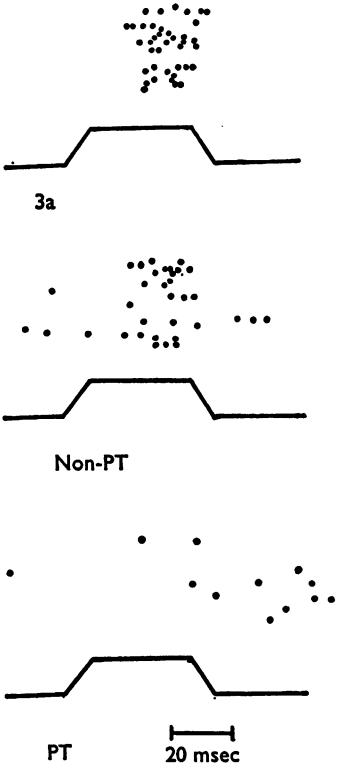


Text-fig. 6. Summary diagram of threshold displacement amplitudes for the various frequencies. The amplitudes (mm) and frequencies (Hz) are plotted on logarithmic scales. Note the similar mean thresholds of PT and non-PT neurones (*A*). *B*, *C*, *D* are plots of individual values for each cell type. Some of the points (marked by ●) represent extrapolated and interpolated values (see text).

Increasing the amplitude of muscle displacement to 2*T* threshold increased the number of evoked spikes in most cells of area 3a and area 4. This increase was observed for all frequencies of sinusoidal stretching and also for step displacements. The factor was not significantly different for the various frequencies; the mean factor (i.e. over all frequency tests) was 2·2 (S.E. of mean ±0·1) for 3a neurones, 1·9 (S.E. of mean ±0·05) for non-PT neurones, and 1·9 (S.E. of mean ±0·06) for PT cells. Thus, a

doubling of input magnitude (displacement) almost doubled the output magnitude (number of spikes of cortical neurones).

Latencies. The histograms of response latencies for the three cell types are illustrated in Text-fig. 9. A distinction is made between responses to low-frequency sinusoidal stretching (*A*) and high-frequency vibration (*B*). A number of cells could not be plotted because it was not possible to measure an exact latency from the dot-display. The shortest latencies

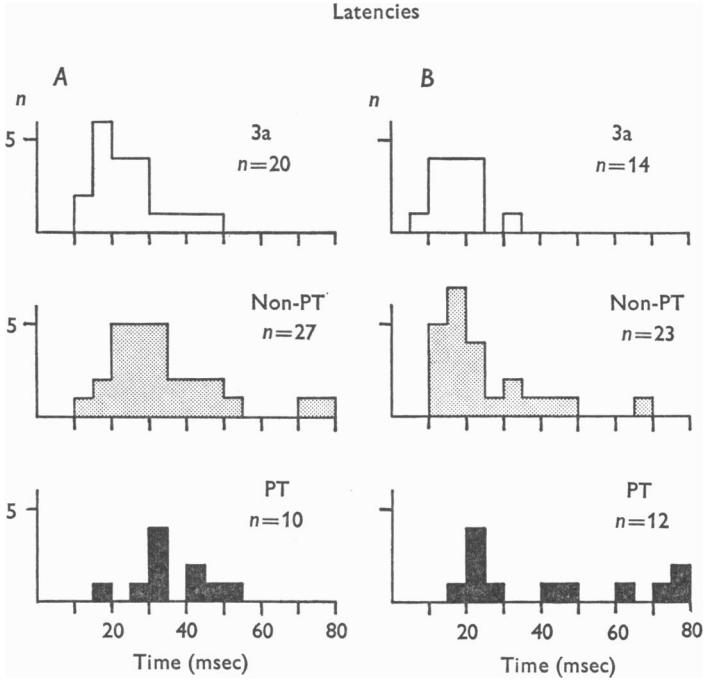


Text-fig. 7. Responses to rhomboidal displacements. Ramp velocities were 8 mm/sec and maximum displacements 80 μm. These stimuli (considered to selectively activate primary but not secondary muscle spindle endings; see text) were capable of activating some of all three cell types.

were observed among the 3a neurones, but non-PT cells had only slightly longer latencies. The longest latencies were among the relatively few PT neurones available for analysis. Statistical treatment with a non-parametric ranking test (Mann–Whitney) revealed that responses of both 3a cells and non-PT cells had significantly shorter latencies to muscle vibration than responses of PT neurones ($P < 0{\cdot}01$). These observations

are compatible with the assumption of a transfer of impulses from area 3a to non-PT neurones and thence to PT neurones.

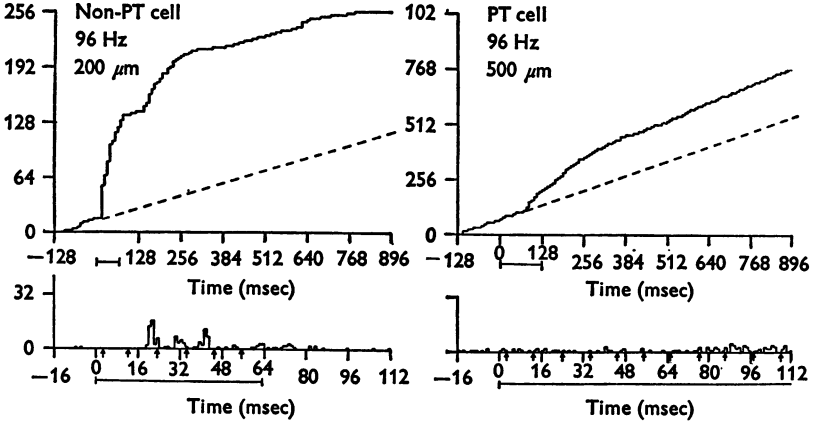
Discharge patterns. It was hoped that the response pattern might give a clue as to which receptor type might be involved in the production of cortical responses. In view of the previous assumption of the role of muscle spindle secondaries (Wiesendanger, 1973), it was expected that



Text-fig. 8. Summary of latency histograms for the three cell types. The latencies were measured from responses obtained at twice threshold amplitudes. *A*, low-frequency sinusoidal stretching (6–12 Hz); *B*, high-frequency sinusoidal stretching (100–300 Hz).

motor cortical cells would respond mainly with tonic responses and 3a neurones with dynamic responses. It turned out, however, that tonic and dynamic responses were seen in both areas, 3a and 4. An annotation of the discharge pattern was made for each cell for responses evoked by low-frequency sinusoidal stretching, high frequency vibration, and step displacements. A short burst (< 30 msec) or a modulation of spike discharges with individual stretch cycles was defined as a dynamic response. Long bursts (> 30 msec) or tonic discharges outlasting the stimulus by several hundred msec were classified as tonic responses. Classification was not always easy because the pattern was sometimes mixed (for in-

stance an initial burst followed by a tonic discharge). The cell was then classified according to the dominant feature. Also, it is likely that the number given for dynamic responses is too low because fine modulations within a long burst may not have been recognized on the dot-display. Low frequency sinusoidal stretching produced dynamic responses most frequently in 3a cells (81 %), somewhat less frequently in non-PT cells (70 %) and PT cells (61 %). With high frequency vibration, 3a cells again



Text-fig. 9. 'Dynamic' and 'tonic' discharge patterns of motor cortical neurones. Cumulative distribution function (top) and post-stimulus time histogram (bottom) of a non-PT cell and a PT cell; 100 stimulus presentations. The entire duration or the first part (lower right) of sinusoidal stretching is indicated by a continuous bar. Peaks of the 96 Hz vibration are marked by arrows. Note different time scales for cumulative distribution function and post-stimulus time histogram. The interrupted lines represent the level of background activity of the neurones. Modulation of the burst response with the individual cycles of stretching became evident in the post-stimulus time histogram of the non-PT neurone. In contrast, the activity of the PT cell increased gradually at a long latency in response to vibration. The tonic response was best revealed in the cumulative distribution function.

displayed dynamic responses most frequently (50 %), followed by non-PT cells (37 %) and by PT cells (38 %). Thus, dynamic responses occurred only slightly less frequently in the motor cortex as compared to area 3a. Tonic response properties were accordingly more often observed in area 4 than in area 3a. The two response patterns were almost equally distributed among the three cell types when step displacements were used. A typical dynamic response pattern of a non-PT cell and a weak tonic response of a PT cell are illustrated as post-stimulus time histograms and as cumulative distribution functions in Text-fig. 9. Purely inhibitory effects were not

seen in cells of area 3a and in only four cells of the motor cortex (one PT, three non-PT cells). However, it was common that burst discharges were followed by a long-lasting inhibition (> 100 msec). A typical short-burst cell of the motor cortex was characterized by a brief excitation followed by a long inhibition. Partial driving of neurones was common at frequencies of 6 Hz, but inhibition prevented driving of the cell already at frequencies of stretching of 12 Hz in most cases (Text-fig. 5).

DISCUSSION

Feed-back from muscle afferents to the motor cortex

The hypothesis of load compensation during voluntary movements *via* a transcortical loop, as proposed by Phillips (1969), requires the demonstration of a muscle spindle feed-back to the motor cortex. In previous experiments, the effects of graded electrical stimulation of muscle afferents from the forelimb on precentral neurones have been described (Wiesendanger, 1973). Since it was found that effective stimuli were always above the threshold for group II afferents, it was tentatively concluded that secondary muscle spindle endings may provide the error signals in a transcortical load compensating circuit. The use of controlled displacements of a forelimb muscle and especially the use of high frequency vibration in the present experiments, however, strongly suggest that primary muscle spindle endings are also involved in a transcortical circuit. The arguments are as follows. (1) The minimal displacement amplitudes required to elicit cortical neurone discharges decreased dramatically as the frequency of sinusoidal stretching was increased. This was not only the case with 3a neurones, known to receive a powerful input from low-threshold muscle afferents (Phillips *et al.* 1971), but also with non-PT and PT neurones of the motor cortex. From studies on first order neurones in the cat (Matthews, 1972), it can be assumed that, for a given stretch amplitude, increasing the frequency of vibration from 100 to 300 Hz, increased the number of activated primary but hardly of any secondary muscle spindle endings. Therefore, it seems reasonable to assume that the increase in sensitivity of cortical neurones accompanying increasing frequency of vibration was due to the increasing sensitivity of primary muscle spindle endings to such stimuli. It is likely that 300 Hz vibration recruited all primary muscle spindle endings of the muscle and thus provided the most powerful input from muscle spindle primaries to the cortex. (2) Dynamic responses to high and low frequency sinusoidal stretching were observed in well over half of the neurones of the motor cortex. (3) Some motor cortical neurones were excited by displacements which were probably below the sensitivity of secondary muscle spindle

endings. (4) In addition, these 'low-threshold neurones' usually responded to rhomboidal stretches of 80 μm amplitude and 8 mm/sec ramp velocity. These stretch stimuli, tested on forelimb muscles of cats, were said to selectively activate primary but not secondary muscle spindle endings (Wong *et al.* 1974).

Cumulatively, these data present a strong argument for an active role of primary (in addition to secondary) muscle spindle endings in the modulation of motor cortical output. In experiments on cats, Murphy *et al.* (1974) and Wong *et al.* (1974) came to the same conclusion. The lower efficacy of step displacements as compared with trains of high frequency vibration furthermore indicates that temporal summation is an important factor in determining the threshold amplitude of higher order neurones. The values of threshold amplitudes for one particular stretch stimulus only are therefore of limited value in ascertaining the receptor involvement at higher levels of the nervous system. Finally, it became evident that high frequency vibration (which probably generated a maximal input from primary muscle spindle endings) is a much more effective means to activate neurones of the motor cortex than repetitive stimulation of muscle nerves below threshold for group II afferents.

Some caution with respect to the 'diagnosis' of receptor-involvement seems appropriate however: high frequency vibration is a powerful stimulus also for Pacinian corpuscles which may be excited by travelling waves from a distance (Mountcastle, Talbot, Sakata & Hyvärinen, 1969). Control experiments with transection of the deep radial nerve made it unlikely that distant activation of receptors in the face or trunk played a role in the present experiments. None of the precautions taken by some investigators (extensive denervation, cutting the humerus, separate support for the vibrator) may, however, preclude the possibility of activating the numerous Pacinian corpuscles of the antebrachial interosseus membrane (Altner, 1971) which is supplied by the deep radial nerve. Destruction or denervation of this membrane, necessary to exclude co-activation of Pacinian receptors, would have been a difficult procedure. The following considerations, however, speak against a major involvement of the Pacinian corpuscles in the results of the present study. According to Burgess & Perl (1973), these receptors are hardly excited at frequencies up to about 50 Hz even with large amplitudes of displacements. In the current study, none of the cells of the motor cortex which was tested with both low-frequency and high-frequency stretches responded to the latter only. More difficult to exclude is the involvement of Golgi tendon organs. Most of the passive stretch amplitudes were well in the range of the tendon organ sensitivity. Furthermore, active tension was produced reflexly (monosynaptic stretch reflex, vibration reflex) which must have

excited many tendon organs. Cortical discharges in response to active tension would have occurred at latencies of at least 30 msec. Harder to explain would be the exquisite frequency-dependent threshold characteristics of the cortical neurones as observed in these experiments. In summary then it appears that muscle spindle secondaries and to some extent also primaries were the most likely receptors responsible for the stretch-evoked responses recorded in the motor cortex. Some contribution from Golgi tendon organs and Pacinian corpuscles can, however, not be excluded.

The pathways for signals from muscle afferents to the motor cortex

Area 3a, at the bottom of the Rolandic fissure, represents the first cortical relay for impulses from primary and possibly also from secondary muscle spindle endings. Whether the responses to muscle stretch observed in the motor cortex are mediated *via* this primary receiving area is not settled. All that can be said at the moment is that transmission from area 3a to motor cortex is compatible with those units in the motor cortex which responded at short latencies and low amplitudes of muscle displacements. Transcerebellar and other complex pathways as demonstrated in cat experiments (Murphy *et al.* 1974) have also to be taken into consideration. Further lesion experiments, combined with electrophysiological recordings, and anatomical studies will be necessary to clarify this question.

Information transmitted to the motor cortex

The present experiments were not designed to test quantitatively the capability of the system to transmit the parameters measured by the receptors (i.e. length, velocity). Several synapses away from the receptors one cannot expect a replica of the receptor response at the motor cortical level. It was therefore rather surprising to find that a twofold increase of the input magnitude (i.e. stretch amplitude) resulted in roughly a twofold increase of the output magnitude (i.e. number of spikes). An investigation of the relationship over a larger range is evidently necessary. Tentatively, however, it may be suggested that a *population* of motor cortical neurones may be capable, by simultaneous averaging (Matthews, 1972), of extracting the position of a given muscle or muscle group. Tonic discharges (similar to the static responses of secondary muscle spindle endings) were less prominent than short burst responses or sine-wave modulated responses. The prominence of dynamic responses may indicate that velocity is a more powerful stimulus feature than position. In this context, it may be mentioned that sudden displacements of a finger in man resulted in a damped oscillation with a better correction of velocity than position

(Tardieu, Tabary & Tardieu, 1968). In those experiments, the e.m.g. responses to the perturbation occurred at a latency indicating a long-loop reflex. If the muscle spindles are functioning as detectors of 'misalignment' between intrafusal and extrafusal muscle fibres in a transcortical servo-loop (Marsden *et al.* 1973; Marsden, 1973), both dynamic and tonic response patterns of motor cortical cells may be expected.

Correlation with behavioural experiments

Sudden perturbations applied to a handle which a monkey has learned to hold in a given position (Evarts, 1973) or to move from target to target (Conrad *et al.* 1974) generated burst discharges in non-PT and PT neurones at latencies which were comparable to those obtained in the present experiments with controlled muscle displacements in anaesthetized monkeys. The perturbations in the behavioural experiments must have excited a large number of various cutaneous and deep receptors. Accordingly, the precentral responses were more prominent and probably more widespread than in the acute experiments. Furthermore, experiments on awake monkeys (Evarts & Tanji, 1974) and in man (Hammond, 1956) revealed the important role of gating systems which, according to the prior instruction, adjust the gain of the long loop reflex. It may be assumed that, under anaesthesia, the input–output gain at the level of the motor cortex is low which then would explain the scarcity of responses of PT cells to stretch stimuli found in the present experiments. It is quite possible that, with a high gain also non-repetitive (i.e. non-vibratory) small displacements would suffice to modify motor cortical output.

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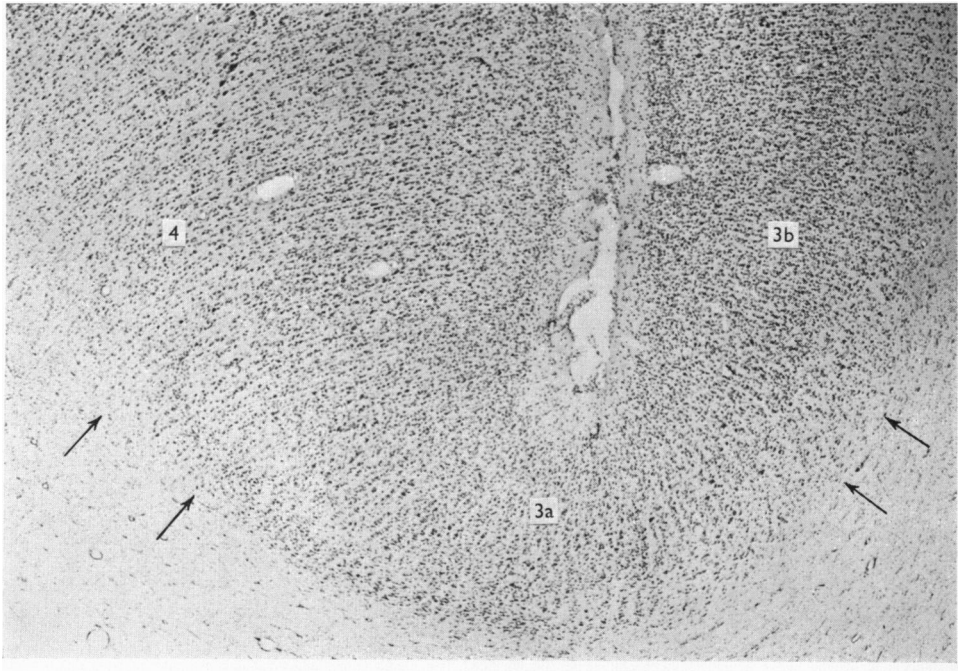
4
3b
3a
1 mm

EXPLANATION OF PLATE

Cytoarchitectonic differentiation of cortical areas 4, 3a, and 3b in the Cebus monkey (see text). Parasagittal section of peri-Rolandic cortex, hand area. Thickness of section 45 μm, stained with thionine. Note appearance of giant pyramidal cells in upper left of area 4. The 3a–4 boundary and the 3a–3b boundary are gradual (transition between arrows).