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SIMULTANEOUS VISUALIZATION OF CORTICAL BARRELS AND HORSERADISH PEROXIDASE-INJECTED LAYER 5b VIBRISSA NEURONES IN THE RAT

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SUMMARY

1. Using diaminobenzidine (DAB) as a chromagen, horseradish peroxidase-injected neurones and cytochrome oxidase-stained barrels were visualized simultaneously in the rat vibrissa cortex. Neurones were initially tested during extracellular recording for responses to whisker deflections. This was followed by intracellular injection of the soma with horseradish peroxidase (HRP) and histological processing to visualize the HRP-stained neurone in an incubation solution which contained, in addition to DAB, cytochrome C for cytochrome oxidase (CO) reaction of the barrels.

2. Recording and intracellular staining were made in layer 5b under urethane anaesthesia. CO-stained barrels were observed in layer 4. Physiologically and morphologically characterized neurones were mostly large pyramidal neurones that responded to more than one whisker and displayed transient-type responses.

3. In tangential sections, the apical dendrite of the HRP-filled neurone was followed from the soma level upward as it ascended through the barrelfield in layer 4. The cross-section of the apical dendrite was found in the periphery of the CO-stained barrel. Using the apical dendrite as a guide, the basal dendritic field of the layer 5b pyramidal neurone was aligned on the pattern of layer 4 barrels. The soma was seen to project basal dendrites in all directions, involving one or two neighbouring barrels/columns.

4. In sixteen neurones examined in tangential sections, a complete spatial tuning map constructed by measuring sensitivity of the neurone to different whiskers could be compared to the basal dendritic field in relation to the pattern of overlying layer 4 barrels. The mean receptive field size in terms of the number of effective whiskers was 5·8 whereas the mean dendritic field size in terms of the number of barrels/columns involved was 2·2. In addition to the well-documented role of intracortical connectivity in elaboration of multi-whisker receptor fields in the cortical neurones, the role played by direct inputs from multi-whisker thalamic ventrobasal neurones was discussed.

INTRODUCTION

A unique feature of the rodent's somatosensory neocortex is the presence of 'barrels' in layer 4 (Woolsey & Van der Loos, 1970). The formation of the barrels is

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under the influence of the sensory inputs from vibrissa follicles, which is consistent with the generally accepted role of layer 4 as the input stage of sensory signal processing in the cortex (Van der Loos & Woolsey, 1973; Steffen & Van der Loos, 1980). Layer 5, on the other hand, is thought to represent the output stage of the cortical signals and a number of physiological studies have revealed convergence of inputs from several whiskers (Simons, 1978, 1985; Ito, 1981, 1985; Chapin & Lin, 1984; Armstrong-James & Fox, 1987).

For layer 4, previous anatomical studies have shown that the dendritic arborization of a neurone whose soma lies within a barrel is limited within the barrel boundary, not radially symmetric but oriented towards barrel centre so as to make contact with the thalamocortical afferents confined in the barrel centre (Woolsey, Dierker & Wann, 1975; Steffen & Van der Loos, 1980). As for layer 5 neurones, it is generally known that they project an apical dendrite through overlying layers perpendicularly and emit radially the basal dendrites in layers 5 and 6, but little is known about the extent and orientation of the dendrites with respect to the overlying barrel pattern. It is interesting to know whether or not these neurones, as expected, always display the lowest threshold of activation to the whisker that is represented by the barrel through which the apical dendritic shaft ascends towards the pial surface. Another interesting question concerns the extent to which their basal and apical dendritic branches in the adjacent barrels/columns, if any, could be compared with the physiologically defined receptive field organization. This issue is relevant to the occurrence of multi-whisker responses in these cortical neurones, since a spread of dendritic arbors into several adjacent barrels/columns might provide one route for convergence.

The extent of dendritic field of pyramidal neurones in the rodent neocortex has been analysed using the technique of intracellular staining (Landry, Wilson & Kitai, 1984; Martin & Whitteridge, 1984). The present experiments were designed to combine intracellular recording and staining of a large layer 5b pyramidal neurone with visualization of the overlying barrel pattern. To do this, an advantage was taken of the common use of a chromagen, diaminobenzidine (DAB) for visualization of cytochrome oxidase (CO)-rich barrels and intracellularly injected horseradish peroxidase (HRP), so that the extent of the dendrites of an output stage neurone can be overlapped on the barrelfield (input pattern).

METHODS

Surgery and preparation

Sixty-eight albino rats of Wistar strain were used in this series of experiments. Under urethane anaesthesia (1·2 g/kg body wt) a small trephine hole was made over the vibrissa area of the somatosensory cortex and the dura was reflected aside or dissected. To minimize the pulsatory movement of the exposed part of the cortex the dura dissection was made as small as possible, and the opening was covered with warm mineral oil.

Whisker stimulation

Whiskers on the right side were cut to 10 mm from the base. Whisker nomenclature by Van der Loos & Woolsey (1973) was adopted: five rows of mystacial vibrissae are labelled, from dorsal to ventral, A to E; whiskers in a row are numbered, from caudal to rostral, 1 through 4–7; caudalmost whiskers straddling two adjacent rows are referred to, from dorsal to ventral, as α, β, γ and δ. The

response properties of vibrissa-sensitive cortical cells were first studied in an extracellular position. Individual vibrissae were deflected with a hand-held probe to note the number of vibrissae that activated the neurone (receptive field (RF) size) together with the whisker that activated the neurone most vigorously (RF centre). Each neurone's sustained/transient response nature was tested during sustained deflection of the central whisker in the most preferred direction. For quantitative analysis of the neurones' response properties, a calibrated whisker stimulator was used. The quantitative stimulation was effectuated by a pen galvanometer, which could be deflected in two ways. Firstly, for the determination of a neurone's velocity threshold, the galvanometer was driven by a short pulse. The deflection velocity and amplitude, as measured at the top of the whisker, were varied conjointly between 5 μm at 1 mm/s and 500 μm at 100 mm/s, and the minimum velocity/amplitude value required to activate a given neurone in half the time (in 50% of the stimulus presentations) was simply termed the velocity threshold and this value was determined separately for different whiskers (see Ito, 1981, 1985, 1988). The sensitivity of the neurone to each whisker was defined as the reciprocal of the measured threshold. Secondly, for quantitative evaluation of the sustained/transient responses of some neurones, the galvanometer was driven by 'ramp-and-hold' waveforms (Fig. 1 lower, Fig. 2). The maximum 'ramp' velocity was 200 mm/s with a constant 'hold' amplitude of 2 mm.

Electrodes and recording

Glass micropipettes were pulled from a fibre-containing capillary, and back-filled with a solution consisting of 4% horseradish peroxidase (HRP) in 0·05 M-Tris buffer which contained 0·2 M-KCl (pH = 8·6). The solution was filtered through a 0·5 μm Millipore filter. Under the microscope the tip of the electrode was brought into contact with a glass rod to make the tip diameter 2 μm. Such an electrode had an *in situ* resistance of 50–100 MΩ.

Intracellular marking and histology

After the response properties of a neurone were noted, 200 ms positive pulses of 3 nA were passed through the electrode at 0·3 Hz to impale the cell membrane. The microelectrode potential fell in steps and when it eventually reached a stable membrane potential of 30–60 mV, the stimulus frequency was switched from 0·3 to 3 Hz and the current was increased up to 6 nA to eject HRP ionophoretically for 3 min. The presence of action potentials was monitored between ionophoretic pulses during the entire period of an injection.

Within 3 h or so of the first staining, the animal was killed by an overdose of Nembutal and perfused through the left ventricle with physiological saline (plus 1000 units of heparin) and then with 1·5% glutaraldehyde and 1·5% paraformaldehyde in 0·5 M-phosphate buffer at pH 7·6. The brain was kept overnight in the same fixative in the refrigerator at 4 °C. The brain was transferred to 10% sucrose in the same buffer and after a while 100 μm frozen sections were cut in a coronal plane, or in a plane tangential to the barrel cortex. The sections were collected first in the phosphate buffer, which was replaced with a diaminobenzidine (DAB) solution in a combination of Adam's intensification method (Adams, 1981) and Wong-Riley's cytochrome oxidase reaction method (Wong-Riley, 1979). The DAB solution contained 30 mg DAB, 10 mg cytochrome C, 0·5 ml of 1% cobalt chloride and 0·5 ml of 1% nickel ammonium sulphate in 50 ml of 0·5 M-phosphate buffer at pH 7·6. The solution was prepared each time before use and filtered through filter paper. After an incubation period of 10–20 min at 37 °C, 0·2 ml of 25% H_2O_2 solution was added and the reaction was terminated after 5–10 min when colouring of the specimen was judged sufficient. Coronal sections were examined to find a section that contained the injected soma. A stained soma and its apical dendrite were usually found in the same 100 μm-thick section. Tangential sections were examined from layer 5b where the injected soma was located, up to layer 4 where the cross-section of the apical dendrite of that neurone was found in the barrelfield. Camera lucida drawings of different tangential sections were first aligned with respect to two landmarks; the cross-section of the apical dendritic shaft and one of the blood vessels in the view field (simple alignment), and then the centre of the soma was moved to the cross-section of the apical dendrite in the layer 4 barrelfield without rotating the drawing sheets (corrected alignment). This is the formal procedure of columnar alignment of the tangential sections, but such a correction was unnecessary or minimal, meaning that sections were cut fairly perpendicular to the barrels/columns as intended. The basal dendritic field around the soma could thus be superimposed on the contour map of the barrels in layer 4.

RESULTS

Seventy-three injections were successful in the sixty-eight animals. Sixty-one neurones were stained by forty injections and recovered in coronal sections, and thirty-six neurones were stained by thirty-three injections and recovered in tangential sections. Two to three neurones could be stained by single injections.

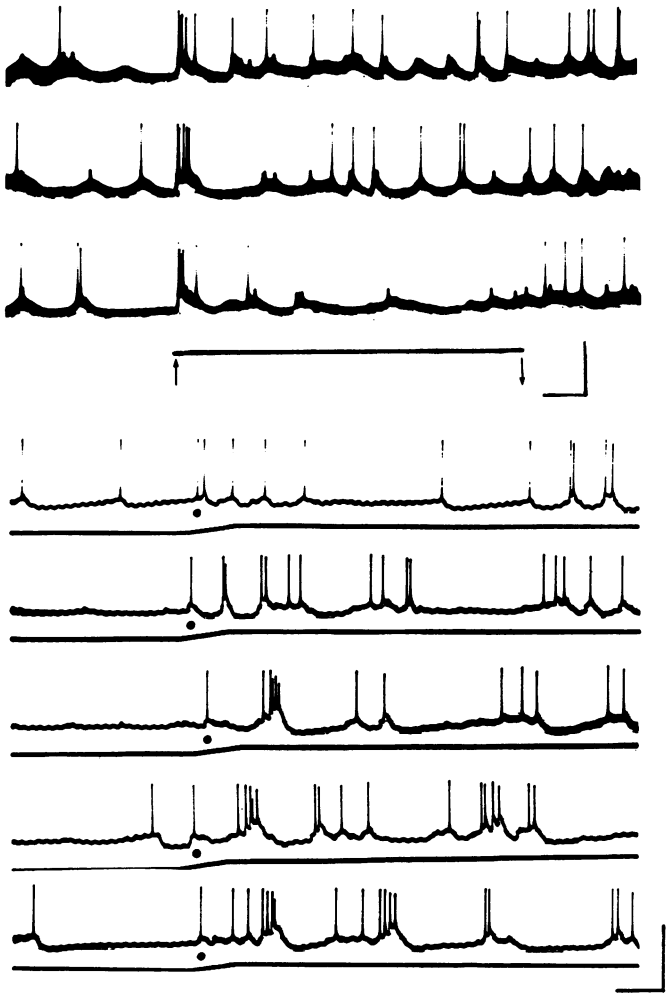

Fig. 1. Two examples of intracellular recording from layer 5 b pyramidal neurones. Upper: three consecutive whisker deflections with an amplitude of 2 mm lasting for 800 ms between up and down arrows. 'Ramp' (onset) velocity was 200 mm/s. Note the transient response. Lower: another neurone. Five consecutive whisker deflections with an amplitude of 2 mm ('ramp' velocity 20 mm/s). This neurone was of purely transient type in the extracellular position. After impalement of the cell membrane, sustained responses emerged as spike bursts during maintained whisker deflection, but transient responses time-locked to the deflection onset are consistent (dots). Calibration: 50 mV and 100 ms.

These apparently reflected dye coupling (Gutnick & Prince, 1981) between neurones because ionophoretic pulses in the extracellular position never resulted in staining of neurones.

Most of the neurones successfully stained with HRP turned out to be of the transient type with a multi-whisker receptive field as examined in the extracellular

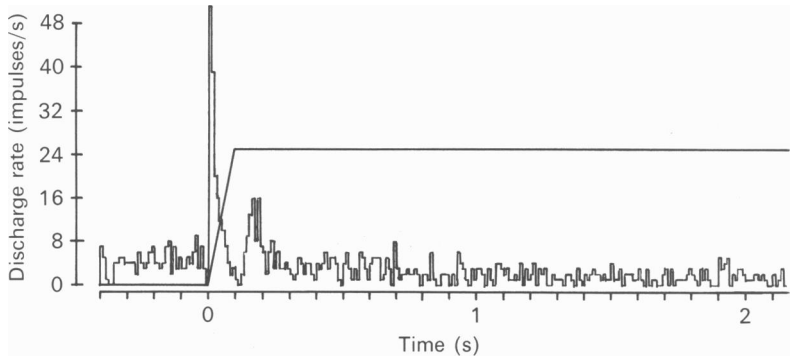

Fig. 2. Peristimulus time histogram of a transient-type layer 5b pyramidal neurone during extracellular recording. The time course of whisker deflection (a plateau amplitude of 2 mm with 20 mm/s 'ramp' velocity) is indicated. Averaged data of 100 responses.

position (Fig. 2). However, once impaled, these neurones tended to display some sustained responses, probably due to slight membrane injury, but the transient nature of the stimulus-locked responses was still evident (Fig. 1). Neurones with a sustained-type response (when examined extracellularly) could be recorded intracellularly only for less than 10 s and had not recovered in histological examination except for the one described below. Of the two waveforms of action potentials described by Simons (1978), neurones with fast spikes were seldom kept long enough in an intracellular position to be stained successfully.

The resting membrane potential of the successfully injected neurones ranged from 30 to 60 mV. The action potential seldom showed overshoots and the amplitude of spikes was the same as of the membrane potential or even smaller by 0–5 mV.

Coronal sections

Figure 3 shows an example of an HRP-stained layer 5b neurone in a coronal section. This neurone had an RF consisting of five whiskers B1, B2, B3, C1 and C2, and centred on whisker C2. The apical dendrite can be followed up to just beneath the cortical surface. The apical dendritic shaft is seen to divide in two or more strands first near the cortical surface (arrow in Fig. 3). Such terminal division of the dendritic shaft occurs at very acute angles, while more proximal branchings are seen to emanate from the dendritic shaft at larger angles in layers 4 and 5a (arrow in Fig. 3, see also Fig. 4*A* and *C*). The basal dendrites are seen to emanate from the soma in five primary branches. The majority of the recovered neurones were large pyramidal neurones (Fig. 4*A*, *B* and *C*). The length of soma ranged from 15–20 μm,

and the width was 10–15 μm. Figure 4*D* shows the only sustained-type neurone that was successfully stained and judged to be a star pyramid. This neurone displayed 'thin' spikes as in Simons (1978).

The extent of basal and apical dendrites of the large pyramidal neurones was quantitatively assessed by placing a template on camera lucida drawings of HRP-

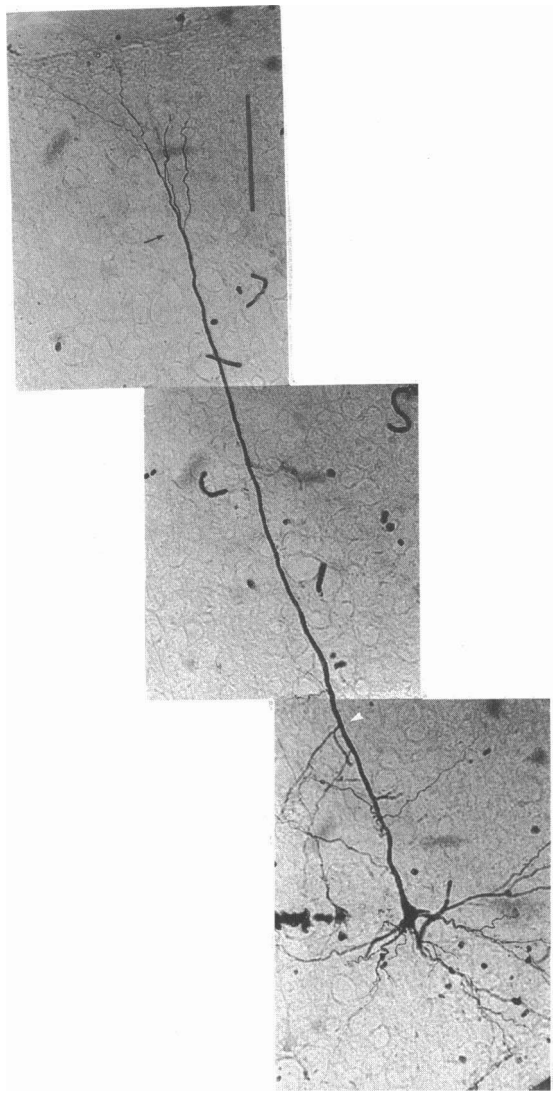

Fig. 3. An HRP-filled layer 5b pyramidal neurone in a coronal section. The bar denotes 100 μm.

labelled neurones. For basal dendrites the number of intersections with each of a series of concentric rings at 20 μm intervals was counted, and for apical dendrites the intersections with each of a set of parallel lines spaced at 20 μm was counted (Fig. 5,

upper). Figure 6 shows this result and indicates that the extent of the apical dendritic branching is smaller than that of basal dendritic branching.

Tangential sections

Figure 7*A* shows the soma and basal dendrites of an HRP-injected pyramidal neurone in layer 5b in a tangential section. Layer 5b neurones in the present study

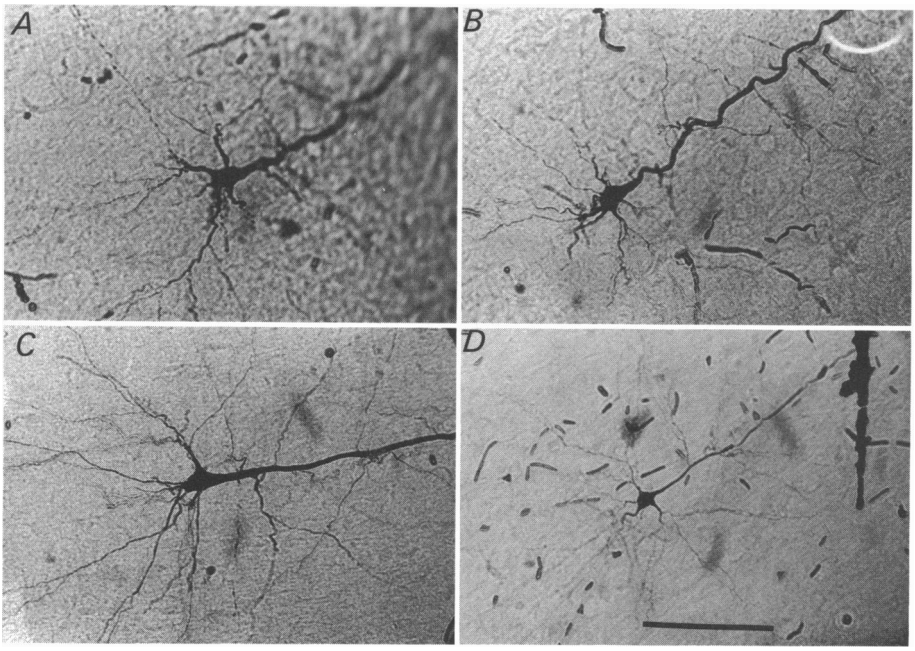

Fig. 4. Four HRP-stained neurones in coronal sections. *A*, *B* and *C*: pyramidal neurones in layer 5b with transient responses. *D*, probably a star pyramid recorded in layer 5b. This neurone displayed 'fast' spikes as in Simons (1978) and the response to whisker deflection was of sustained type. The bar in *D* denotes 100 μm and applies also to *A*, *B* and *C*.

extended basal dendrites in four or five primary branches at about equally spaced angles. The entire dendritic arborization was evenly distributed around the soma, and there was no indication that any particular direction was preferred (radially symmetric). The apical dendrite can be followed in successively more superficial sections (Fig. 7*B–E*). Figure 7*F* is a lower-power magnification of Fig. 7*E* and shows that the apical dendrite (arrow) passes through the peripheral aspect of the D3 barrel. None of the neurones in the present sample were found to extend their apical dendrite through the septum between barrels.

To illustrate the columnar relationship between the barrel and the injected neurones, it is appropriate to align the centre of the soma of a neurone (in a deeper section) on the cross-section of its apical dendritic shaft appearing in the layer 4 barrelfield (in a more superficial section) (see Methods). Figure 8 shows an example

of one of such dendritic maps. For the purpose of the present analysis, the barrel/column in which the soma was located was referred to as the 'home' barrel (A4 barrel in this case). The nearest barrels on the same row and on the same arch were 'first-order' and 'second-order' neighbour barrels respectively, the 'third-

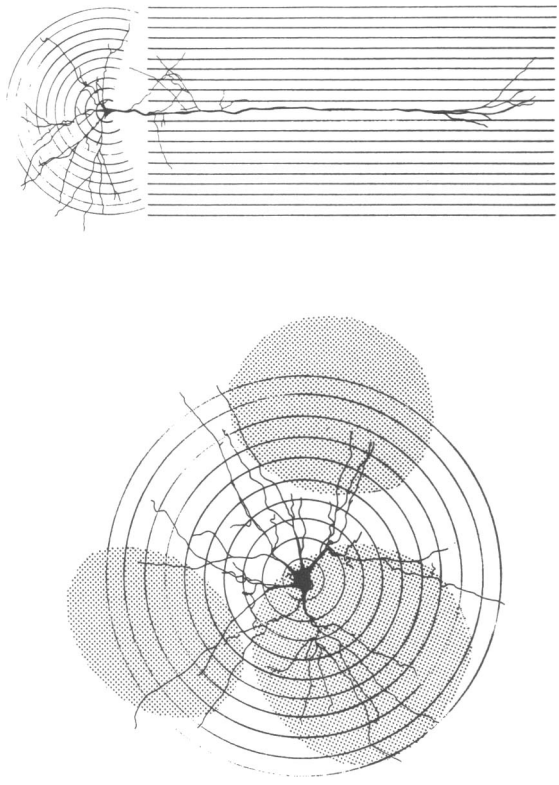

Fig. 5. Method of counting the dendritic intersections with lines or rings according to Sholl (1953). Upper: basal dendrites (left) and apical dendritic branches (right) in a coronal section. Lower: basal dendrites in a tangential section.

order' neighbour barrel being the barrel facing the last two (i.e. orthogonal to the home barrel). The inset is a quantitatively-defined RF (receptive field) map constructed during physiological recording and shows that this neurone responded to the whiskers A4, A3 and B4. The sizes of filled circles indicate the sensitivity of the neurone to deflection of individual whiskers (see figure legend). In this neurone, the dendritic field organization was intimately correlated with the RF map defined physiologically. The soma located in the periphery of the barrel A4 gives rise to basal dendritic arborization predominantly into barrel A4 ('home' barrel) and the septum (S), but some distal branches are seen within the barrels A3 and B4 as well. While the soma was associated with the barrel A4, the RF was centred on the whisker A3. In this respect this neurone was exceptional (see later).

Neurones illustrated in Figs 9 and 10 are examples of the type of neurones that are

more commonly observed. Both neurones responded to eight whiskers but their dendritic fields involved only two barrels (home and first-order neighbour). In both cases the soma was located in the peripheral aspect of the barrel that was anticipated from the RF centre (the whisker of the lowest threshold).

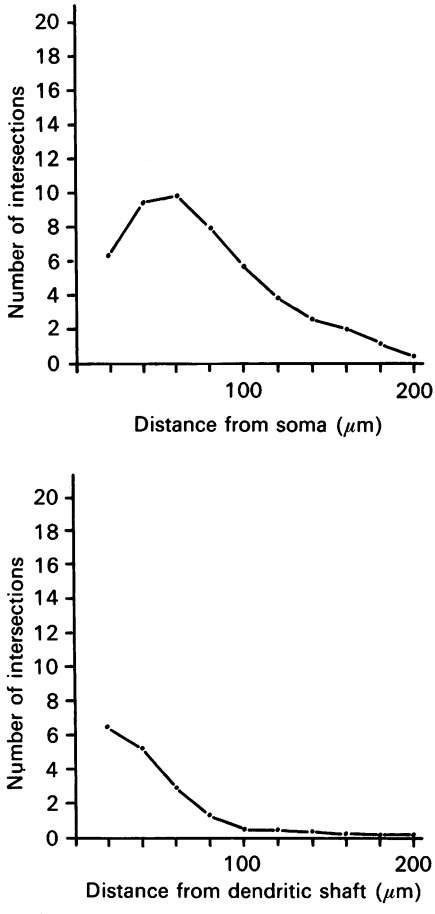

Fig. 6. The number of intersections between dendritic branches, and rings (basal dendrites, upper) or lines (apical dendrites, lower) at 20 μm intervals. Averaged data from nineteen neurones in coronal sections.

A quantitative analysis of the tangential sections, based on Sholl's method (1953), involved superimposing a series of concentric rings at intervals of 20 μm, centred on the soma, and to count the number of intersections of basal dendrites with the rings within each barrel and between the barrels (Fig. 5, lower). Figure 11 summarizes the results obtained from sixteen well-stained neurones. Intersections were clearly most frequent in the 'home' barrel and many intersections were counted in the septum, but some intersections were also found in the neighbouring barrels. Statistical comparisons (ANOVA) showed that the number of intersections in the 'home' barrel

was greater than that in the septum, and the intersections occurring in the septum were much more numerous than the sum of intersections in all neighbouring barrels. Although statistically not significant, the 'first-order' neighbouring barrel contained more intersections than the 'second-order' neighbouring barrel which, in turn, contained more intersections than the 'third-order' neighbouring barrel (Table 1).

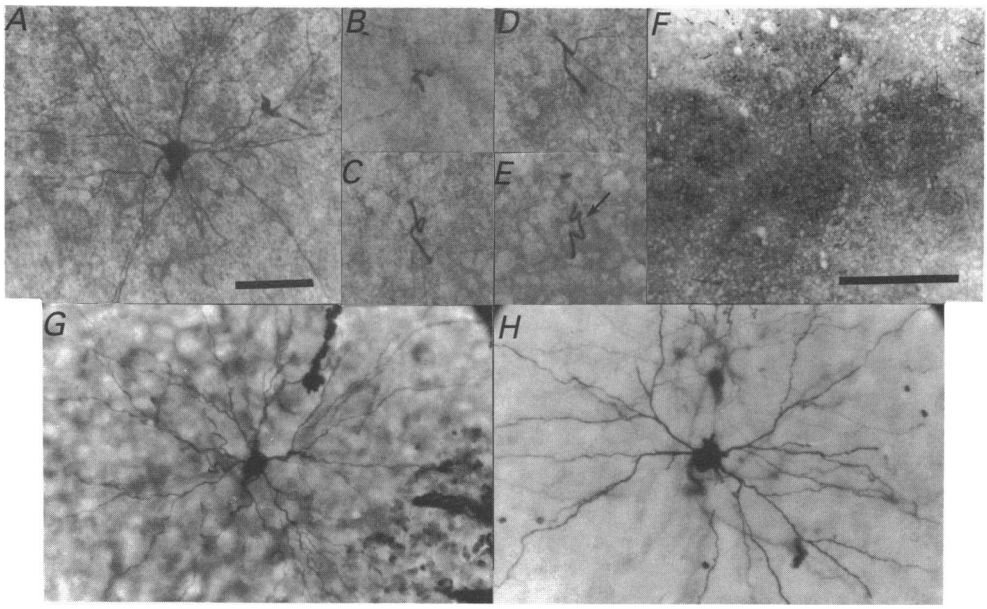

Fig. 7. Tangential views of HRP-injected layer 5b neurones. Examples of three neurones (*A–F*, *G*, *H*, respectively) are given. *A–F*, a series of tangential sections at 100 μm. *A* shows the level of soma and basal dendrites, and in this section the initial part of the apical dendritic shaft is seen as an appendage below the soma. Its ascending apical dendrite can be followed in successively more superficial sections (*B–D*) until it passes through a barrel in layer 4 (*E*, *F*). *F* is a low-power view of the section including *E*. Arrows in *E* and *F* point to the identical portion. Note that in *F* the apical dendrite ascends through the peripheral aspect of barrel D3 (at the top). In this section barrels D3, D4, C3 and C4 are visible (not labelled). The specimen is from the left hemisphere, with rostral to the left and medial upwards. The calibration bar in *A* denotes 50 μm and applies to the other frames except in *F* where the bar is for 500 μm. *G* and *H* show two other HRP-injected neurones at the level of the soma. Note the evenly spaced distribution of basal dendritic arbors.

The 'home' barrels for the sixteen neurones were A2, A3, A4, B3, B4, C2, C3, C4, C5, D2, D3, D5, with C3 and C4 barrels occurring twice and with the D3 barrel occurring three times. Of the sixteen neurones analysed in tangential sections, the somata were found in the barrel/column that was correct for the whisker with the lowest threshold except for two neurones including the one presented in Fig. 8. Figure 12 summarizes the relation between the anatomically defined dendritic field and the physiologically defined receptive field features. For each of the sixteen neurones, a spatial tuning map is depicted as an array of filled circles each representing the sensitivity (reciprocal of velocity threshold) of a neurone to different

whiskers, and the barrel/columns found to contain dendritic intersections are encased by rectangles. The following findings are to be noted: (1) the receptive field was generally larger than the 'dendritic field', and (2) the former generally included the latter. In terms of the number of whiskers or barrels, the mean receptive field size

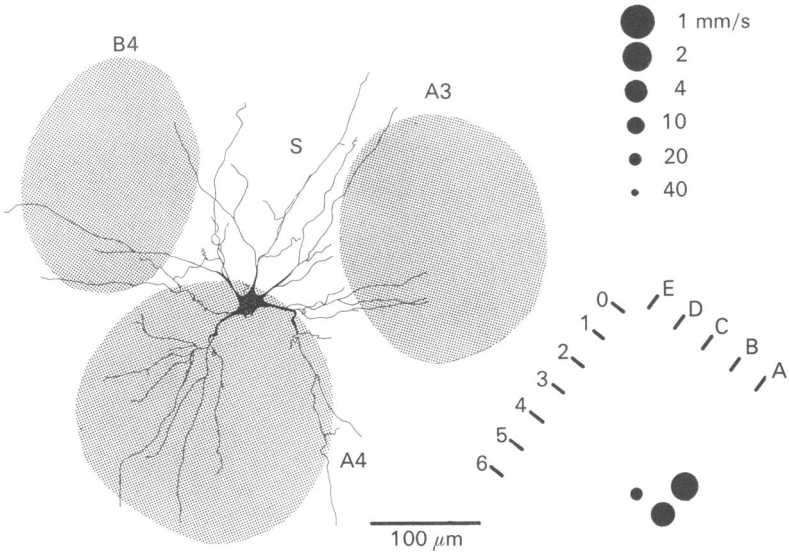

Fig. 8. Composite drawing of a layer 5b neurone and barrels in layer 4 as seen in a tangential plane. The barrel below which the soma was located was termed 'home' barrel (A4). The nearest barrel in the row to which the 'home' barrel belonged (row A in this case) was termed the '1st-order' neighbouring barrel (A3), the nearest barrel in the arch to which the 'home' barrel belonged (arch 4 in this case) being the '2nd-order' neighbouring barrel (B4). The '3rd-order' neighbouring barrel was orthogonal to the 'home' barrel and in this case it was barrel B3 (not shown). S denotes the septum between the barrels. Camera lucida drawings (on tracing paper) of adjacent sections from layer 5b to layer 4 were aligned by referring to the apical dendrite and a blood vessel. Inset at right, lower: the spatial tuning map of this neurone. The whiskers forming the receptive field of this neurone are indicated by filled circles of different sizes according to the sensitivity. The sensitivity was defined as the logarithm of the reciprocal of the measured velocity threshold with respect to 100 mm/s and is expressed as the radius of each filled circle. The radius is linear to log (100/VT) where VT is the velocity threshold in terms of mm/s. Threshold measurements were made using short-pulsed deflection of individual whiskers. Note that the dendritic field and the receptive field involve the same whiskers in this particular neurone. Key to velocity thresholds at right, upper.

was $5{\cdot}8 \pm 3{\cdot}5$ (S.D.; $n = 16$) and the mean dendritic field size was $2{\cdot}2 \pm 0{\cdot}7$. The mean velocity measured for the RF centre was $15{\cdot}2 \pm 21{\cdot}9$ mm/s.

As for the orientation of dendritic arborization, layer 5b neurones appeared to emit basal dendrites evenly (as seen in tangential sections) without any directional preferences (Fig. 7*A*, *G* and *H*). This was tested again by a method similar to Sholl's, but for this purpose a reference line was drawn, passing the somal centre and perpendicular to the line between this somal centre and the centre of the 'home' barrel, and counting the number of intersections with concentric rings separately for

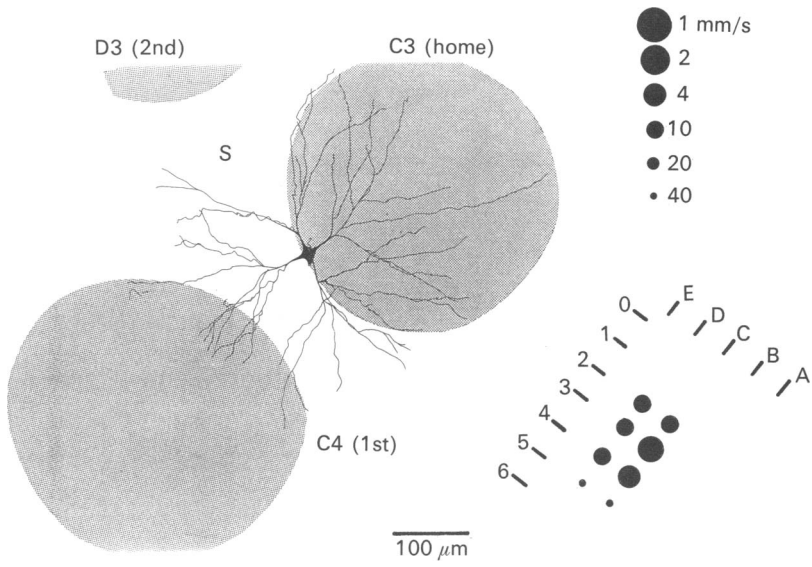

Fig. 9. A typical layer 5b pyramidal neurone with a dendritic field which is much smaller than the receptive field.

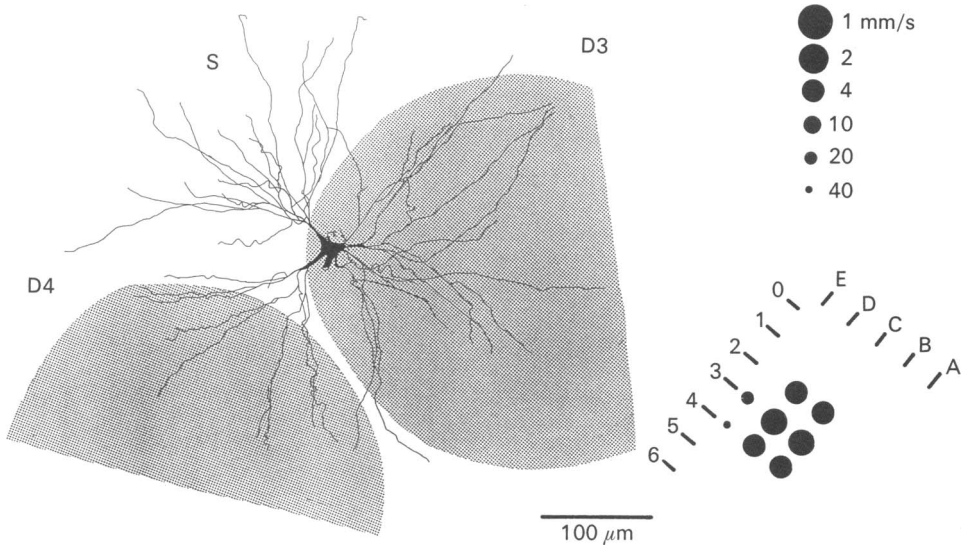

Fig. 10. Similar to Fig. 9 but from another neurone.

each of the hemifields on either side of the reference line. On average, the number of intersections in the centre-oriented and septum-oriented hemifields were 90·6 and 82·4 respectively; this difference was not significant. Moreover, when the number of intersections was considered separately for each of the concentric rings, no difference was detected between the two sides at any value of radius.

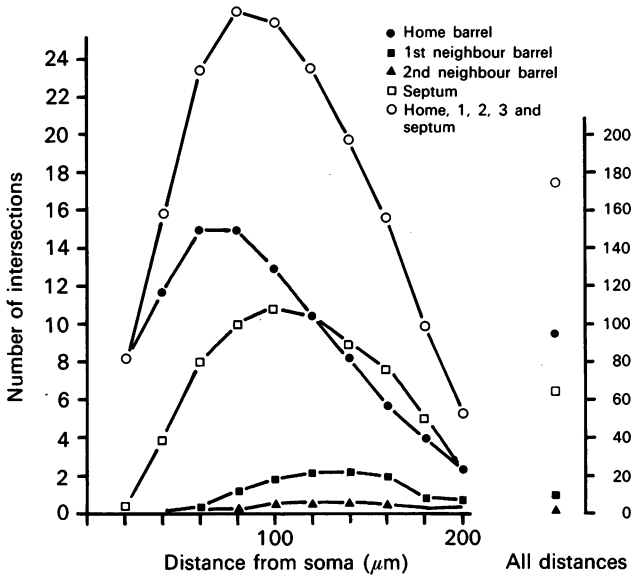

Fig. 11. Sholl analysis of the basal dendritic field of HRP-injected layer 5b neurones. Averaged data from sixteen well-stained neurones examined in tangential sections. A set of concentric rings with 20 μm intervals were centred on the soma and intersections of dendrites were counted for each radius (abscissa). This analysis was done on composite drawings of basal dendrites and barrels such as shown in Fig. 8 (see also Fig. 5, lower) so that the degree of the dendritic extension into different barrels and the septum can be expressed in terms of the number of intersections. The curve defined by open circles is what is usually seen in a Sholl analysis. Its shape and magnitude is similar to those obtained in many Golgi studies of pyramidal neurones of mammalian cortices. The numbers of intersections occurring in the 'home' barrel, septum, '1st-order' and '2nd-order' neighbouring barrels at increasing eccentricity from the soma are indicated by symbols (key to the symbols in inset). Intersections in the '3rd-order' neighbouring barrel are not shown. At right (All distances), the numbers of intersections at different rings were added up and shown separately for different regions (note the different scale factor at right end). For each of sixteen neurones the numbers of intersections were calculated for individual barrels and septum and subjected to statistical tests (see Table 1).

TABLE 1. Partition of basal dendritic field into different zones

	Number of intersections		Significance of differences of means (multiple comparisons)			
					Neighbouring barrels	
Zones	Mean ($n = 16$)	Percentage	Home	Septum	1st	2nd
Home barrel	93·2	53·81	—	—	—	—
Septum	67·1	38·76	< 0·01	—	—	—
Neighbouring barrels						
1st order	11·1	6·39	< 0·001	< 0·001	—	—
2nd order	1·4	0·83	< 0·001	< 0·001	n.s.	—
3rd order	0·4	0·22	< 0·001	< 0·001	n.s.	n.s.
Total	173·2	100·0				

The overall difference among zones (barrels/septum) was found highly significant by analysis of variance ($F = 65{\cdot}63$, d.f. $= 4/75$, $P \ll 0{\cdot}00001$). n.s., not significant.

DISCUSSION

Morphology of layer 5b transient-type neurones in the rat barrel cortex

Of the two response types (transient and sustained) found during extracellular recordings, only the transient-type neurones could be successfully stained with HRP

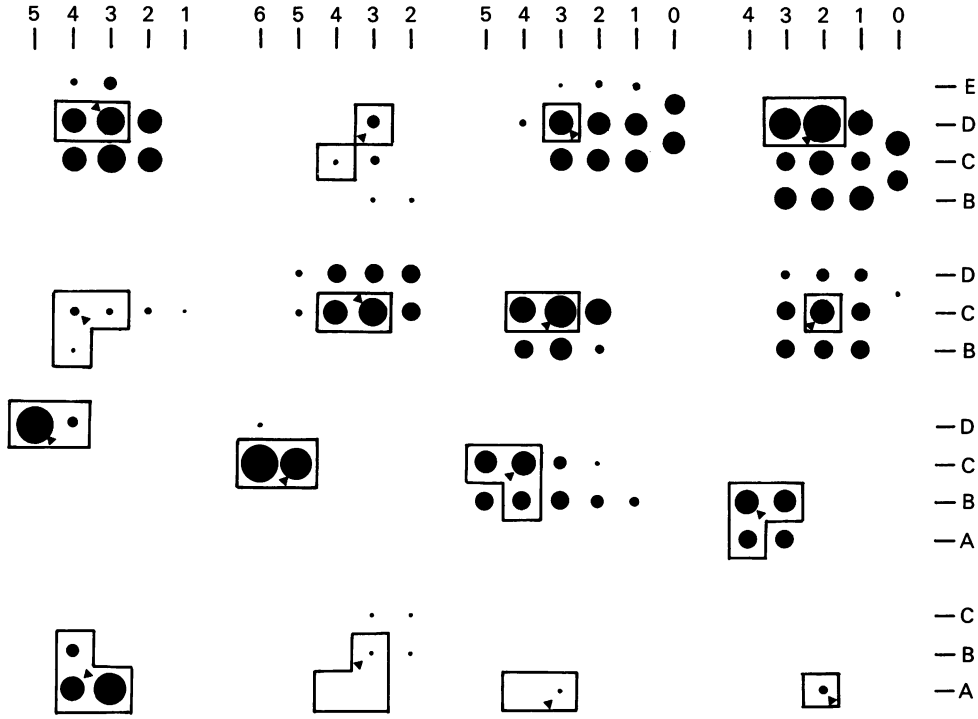

Fig. 12. Comparison between basal dendritic field and receptive field. Data from sixteen neurones are displayed roughly in the order of the position of the barrel/column in which the soma was found. Dorsal barrels up and caudal barrels to right. Physiological data are expressed by filled circles as in Fig. 8. Morphological data are shown by boxes where basal dendrites were found. The position of the soma with respect to the overlying barrel is indicated by a triangle placed in the corresponding box on a quadrant basis.

and proved to be large pyramidal neurones. Successfully stained neurones displayed 'broad' spike potentials except for one neurone (Fig. 4*D*). In this respect, it is recalled that Simons (1978) described two types of spike forms: regular spikes and fast spikes; and McCormick, Connors, Lighthall & Prince (1985) described three types: bursting, regular- and fast-spiking. On the basis of electrophysiological and morphological findings, the class of neurones presently analysed would be identified as the regular spikes in the former, and bursting and regular spiking neurones in the latter paper.

The sustained type of neurones, on the other hand, were occasionally recorded extracellularly and penetrated with the electrode briefly, but could not be held long enough to be filled with HRP. This shows that they are probably smaller in somal

size than the transient-type neurones that are relatively easily identifiable. This interpretation is consistent with the observation that the sustained-type neurones were mostly fast-spiking, a phenomenon which is generally attributed to the small size of the somata.

The position of vibrissa-responding neurones in relation to cortical barrel/column

The present method, a modified combination of Adams HRP intensification (Adams, 1981) and Wong-Riley's CO staining (Wong-Riley, 1979), simultaneously visualizes HRP-injected neurones and whisker-related barrels in the rat somatosensory cortex. The advantage of this method is twofold: (1) the same neurones are examined physiologically and morphologically. HRP injection into the cell soma allows detailed visualization of dendritic structure; (2) using DAB as chromagen, CO-stained barrels and HRP-stained neurones are reacted and visualized at the same time. The apical dendritic shaft can be used as the guide for columnar alignment.

Fourteen out of sixteen pyramidal neurones had RFs centred on the whisker that was represented by the barrel through which the apical dendrite ascended. Similarly, Armstrong-James & Fox (1987) reported that fifty-five of sixty-four layer 4 neurones were driven most effectively by the whisker that was histologically 'appropriate' for the barrel location.

Many previous authors examined the relationship between the distribution of neurones and the structure of the barrelfield. These studies included a variety of methods to specify different morphological and immunochemical types of neurones in relation to the barrel pattern. Some immunoreactive classes of neurones have been found preferentially along the walls of barrels (Kristt & Waldman, 1986; Erzurumlu, Jhaveri & Benowitz, 1990). The present sample of layer 5b neurones were found deep in the marginal zone of a CO-reacted barrel (Fig. 13). This finding is in good agreement with a previous observation in the mouse that retrogradely labelled neurones of layer 5 from cortical and subcortical structures are in register preferentially with the periphery of the barrels (Crandall, Korde & Caviness, 1986). The present analysis was based on the assumption that apical dendrites are in parallel to the columns. A possibility that cannot entirely be rule out is that the somata of many of the labelled neurones may have been more centred beneath the barrel than would have been expected from the present method of columnar alignment. For example, Escobar, Pimienta, Caviness, Jacobson, Crandall & Kosik (1986) have shown that apical dendrites of deep pyramidal cells in the mouse barrel cortex bundle together as they pass through layer 4. Fascicles of apical dendrites may be displaced towards the sides of the barrels. However, the error in the estimate of the soma–barrel relation arising from the coalescence of apical dendrites seems to be minimal in view of Fig. 1 and many other HRP-stained neurones in coronal sections.

None of the present samples of neurones were found in relation to the very core of the barrel/columns or the septum between the barrels. It is possible that neurones in these zones are not easily activated by the usual methods of whisker stimulation, and/or are small in size to be penetrated with the electrodes currently used.

Comparison of spatial tuning map with basal dendritic field

Quantitative analysis of basal dendrites was undertaken for a single 100 μm tangential section that included the cell body in layer 5b. Some of these basal dendrites were seen to extend into other layers, especially into layer 6 (deeper

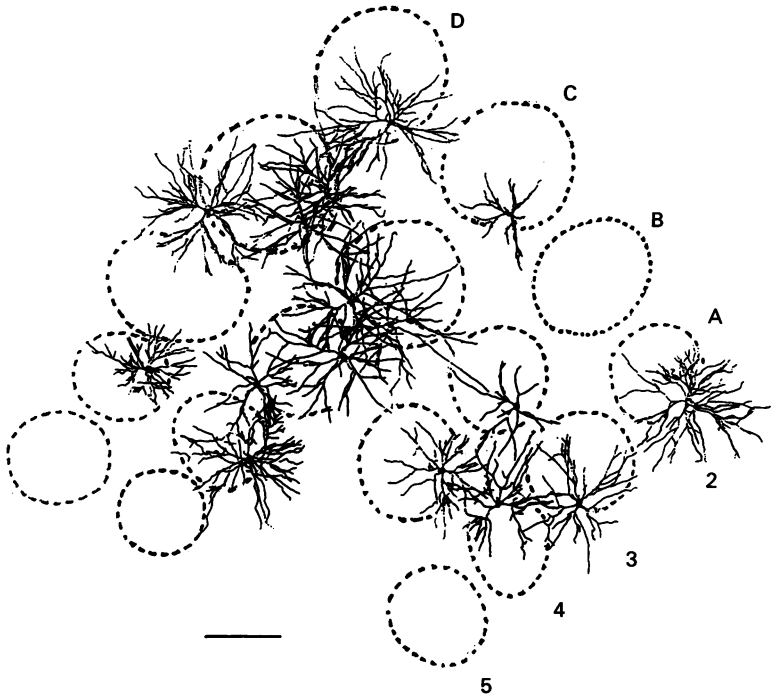

Fig. 13. Photographic superposition of the sixteen neurones examined in tangential sections. The somata were positioned on a contour map of a typical pattern of CO-stained barrels. Therefore, the spatial relation between the barrels and the basal dendrites is slightly different from the actual picture in individual cases. This is only to demonstrate the position of the somata with respect to the barrel pattern. Bar: 200 μm.

sections), but they were not incorporated in the camera lucida drawing for analysis. The error due to this omission may have underestimated the dendritic field radius only minimally since horizontally oriented basal dendrites (that were not missed) more or less determined the extent of the basal dendritic arborization. A second concern was a possible spread of movement of a whisker transmitted to the base of the neighbouring whiskers within the mystacial pad. This may cause an overestimation of the receptive field size. However, there are reportedly few mechanical interactions in the periphery unless the neighbouring whiskers are held in place (Simons, 1985).

A clear difference between layer 5b neurones in this study and layer 4 barrel neurones in the previous Golgi studies is remarkable in the extent and orientation of dendritic arborization (Woolsey *et al.* 1975; Steffen & Van der Loos, 1980; Simons & Woolsey, 1984; Greenough & Chang, 1988). While layer 4 neurones, with the soma

on the side of a barrel, project the dendrites in the hemifield that includes the centre of the barrel, layer 5b neurones extend the basal dendrites in all directions without any orientation preference. Neurones in layer 5b of the vibrissa cortex have been shown to respond to more than one whisker (Simons, 1978, 1985; Ito, 1981, 1985; Chapin & Lin, 1984; Armstrong-James & Fox, 1987). The question was asked if the multivibrissal receptive field of a layer 5b neurone could be explained by the extension of basal dendrites into neighbouring barrels/columns. The sixteen neurones in Fig. 12 had an averaged basal dendritic field size of 2·2 and an averaged receptive field size of 5·8 in terms of the number of whisker/barrels. The classical view that the thalamocortical relay neurones generally respond to only one whisker (e.g. Waite, 1973) could hardly explain this difference between the morphological and physiological data. Note also that the extent of dendritic branchings along the apical dendritic shaft is even smaller (Fig. 6, apical *vs.* basal). Recently, the present author (Ito, 1988) has reported that transient- and sustained-type neurones in the thalamic ventral posteromedial nucleus have a mean RF of 4·3 and 1·6 respectively in terms of the number of whiskers (see also Simons & Carvell, 1989; Sugitani, Yano, Sugai & Ooyama, 1990; Armstrong-James & Callahan, 1991). The apparent discrepancy between the receptive field size and the dendritic field size can be explained by supposing that the multi-whisker transient-type thalamic neurones project to the multi-whisker transient-type pyramidal neurones in layer 5b. This discussion was necessary since there was some uncertainty as to the thalamocortical nature of the ventrobasal neurones that displayed multi-whisker responses in the previous study (Ito, 1988).

An alternative, though not incompatible, view relates to the widely accepted role of intracortical connectivity in convergence of multiple whisker information to single cortical neurones. Simons (1978) proposed that progressively larger receptive fields are elaborated as intracortical processing of information proceeds from layer 4 and deep layer 3 to more superficial layers and then to deep layers. Bernardo, McCasland, Woolsey & Strominger (1990) provided anatomical evidence of heavy interconnections of barrel-related columns above and below layer 4. More recently, Armstrong-James, Callahan & Friedman (1991) have shown that a lesion in a barrel results in a deficit in response magnitude of multi-whisker neurones in the neighbouring barrel to stimulation of the whisker represented by the lesioned barrel. All these data suggest that multi-whisker receptive fields are elaborated by intracortical transformation of vibrissa information about spatial environment.

Termination of thalamocortical afferents has been studied in detail by numerous authors (Bernardo & Woolsey, 1987; Jensen & Killackey, 1987; Chmielowska, Carvell & Simons, 1989). Axons arborize most profusely in layer 4, and form synapses with dendrites there but the somata of origin could be in layer 4 or in other cortical layers (White, 1979). Moreover, ramifications of axon terminals have also been observed in other layers. Relevant to the present data on the basal dendritic expansion of the layer 5b pyramidal neurones is the result of Chmieloska *et al.* (1989) who have shown that the pattern of anterograde labelling following thalamic injection of HRP is barrel-like also in layers 5b and 4. Laminar analysis of cortical potentials to ventrobasal stimulation revealed two distinct early sinks in layer 4 and deep layer 5 (Agmon & Connors, 1991). It is tempting to assume that a typical layer

5b pyramidal neurone extends the basal dendrites into one or two neighbouring columns, receives overlapping, multi-vibrissal information of 4·3 whiskers per column (Ito, 1988) from the ventroposterior medial nucleus (VPM) and thus becomes responsive to 5·8 whiskers (the present study). Moreover, Armstrong-James & Callahan (1991), based on their finding of a mismatch of latencies for surround- and centre-receptive fields in the thalamus and cortex, reasoned that cortical layer 4 barrel neurones are not recipients of inputs from multiple whisker neurones in VPM. Cortical pyramidal neurones then come up as a candidate of cell types on which multiple whisker VPM information ends directly. On the other hand, thalamic posterior (POM) neurones are also known to respond to more than one whisker (Belford, Killackey, Chiaia & Rhoades, 1988), and project predominantly to the septum between the barrels (Koralek, Jensen & Killackey, 1988) to which about 40% of the apical dendritic field of an averaged layer 5b pyramidal neurone belonged (Table 1). The relative contribution of VPM and POM inputs in construction of multiple whisker fields in cortical neurones needs to be assessed in future studies.

REFERENCES

Adams, J. C. (1981). Heavy metal intensification of DAB-based HRP reaction product. *Journal of Histochemistry and Cytochemistry* **29**, 775.

Agmon, A. & Connors, B. W. (1991). Thalamocortical responses of mouse somatosensory (barrel) cortex in vitro. *Neuroscience* **41**, 365–379.

Armstrong-James, M. & Callahan, C. A. (1991). Thalamo-cortical processing of vibrissal information in the rat. II. Spatiotemporal convergence in the thalamic ventroposterior medial nucleus (VPm) and its relevance to generation of receptive fields of S1 cortical 'barrel' neurones. *Journal of Comparative Neurology* **303**, 211–224.

Armstrong-James, M., Callahan, C. A. & Friedman, M. A. (1991). Thalamo-cortical processing of vibrissal information in the rat. I. Intracortical origins of surround but not centre-receptive fields of layer IV neurones in the rat S1 barrel field cortex. *Journal of Comparative Neurology* **303**, 193–210.

Armstrong-James, M. & Fox, K. (1987). Spatiotemporal convergence and divergence in the rat S1 'barrel' cortex. *Journal of Comparative Neurology* **263**, 265–281.

Belford, G. R., Killackey, H. P., Chiaia, N. L. & Rhoades, R. W. (1988). Characterization of neurones in the ventral posterior and posterior nuclei of the rat, using physiological, light and electron microscopic techniques. *Society for Neuroscience Abstracts* **14**, 50.4.

Bernardo, K. L., McCasland, J. S., Woolsey, T. A. & Strominger, R. N. (1990). Local intra- and interlaminar connections in mouse barrel cortex. *Journal of Comparative Neurology* **291**, 231–255.

Bernardo, K. L. & Woolsey, T. A. (1987). Axonal trajectories between mouse somatosensory thalamus and cortex. *Journal of Comparative Neurology* **258**, 542–564.

Chapin, J. K. & Lin, C.-S. (1984). Mapping the body representation in the SI cortex of anesthetized and awake rats. *Journal of Comparative Neurology* **229**, 199–213.

Chmielowska, J., Carvell, G. E. & Simons, D. J. (1989). Spatial organization of thalamocortical and corticothalamic projection systems in the rat SmI barrel cortex. *Journal of Comparative Neurology* **285**, 325–338.

Crandall, J. E., Korde, M. & Caviness, V. S. Jr (1986). Somata of layer V projection neurons in the mouse barrelfield cortex are in preferential register with the sides and septa of the barrels. *Neuroscience Letters* **67**, 19–24.

Erzurumlu, R. S., Jhaveri, S. & Benowitz, L. I. (1990). Transient patterns of GAP-43 expression during the formation of barrels in the rat somatosensory cortex. *Journal of Comparative Neurology* **292**, 443–456.

Escobar, M. I., Pimienta, H., Caviness, V. S. Jr, Jacobson, M., Crandall, J. E. & Kosik,

K. S. (1986). Architecture of apical dendrites in the murine neocortex: dual apical dendritic systems. *Neuroscience* **17**, 975–989.

GREENOUGH, W. T. & CHANG, F.-L. F. (1988). Dendritic pattern formation involves both oriented regression and oriented growth in the barrels of mouse somatosensory cortex. *Developmental Brain Research* **43**, 148–152.

GUTNICK, M. J. & PRINCE, D. A. (1981). Dye coupling and possible electrotonic coupling in the guinea pig neocortical slice. *Science* **211**, 67–70.

ITO, M. (1981). Some quantitative aspects of vibrissa-driven neuronal responses in rat neocortex. *Journal of Neurophysiology* **46**, 705–715.

ITO, M. (1985). Processing of vibrissa sensory information within the rat cortex. *Journal of Neurophysiology* **54**, 479–490.

ITO, M. (1988). Response properties and topography of vibrissa-sensitive VPM neurons in the rat. *Journal of Neurophysiology* **60**, 1181–1197.

JENSEN, K. F. & KILLACKEY, H. P. (1987). Terminal arbors of axons projecting to the somatosensory cortex of the adult rat. I. The normal morphology of specific thalamocortical afferents. *Journal of Neuroscience* **7**, 3529–3543.

KORALEK, K.-A., JENSEN, K. F. & KILLACKEY, H. P. (1988). Evidence for two complementary patterns of thalamic input to the rat somatosensory cortex. *Brain Research* **463**, 346–351.

KRISTT, D. A. & WALDMAN, J. V. (1986). Late postnatal changes in rat somatosensory cortex. *Anatomy and Embryology* **174**, 115–122.

LANDRY, P., WILSON, C. J. & KITAI, S. T. (1984). Morphological and electrophysiological characteristics of pyramidal tract neurons in the rat. *Experimental Brain Research* **57**, 177–190.

MCCORMICK, D. A., CONNORS, B. W., LIGHTHALL, J. W. & PRINCE, D. A. (1985). Comparative electrophysiology of pyramidal and sparsely spiny stellate neurons of the neocortex. *Journal of Neurophysiology* **54**, 782–806.

MARTIN, K. A. C. & WHITTERIDGE, D. (1984). Form, function and intracortical projections of spiny neurones in the striate visual cortex of the cat. *Journal of Physiology* **353**, 463–504.

SHOLL, D. A. (1953). Dendritic organization in the neurons of the visual and motor cortices of the cat. *Journal of Anatomy* **87**, 387–406.

SIMONS, D. J. (1978). Response properties of vibrissa units in rat SI somatosensory neocortex. *Journal of Neurophysiology* **41**, 798–820.

SIMONS, D. J. (1985). Temporal and spatial integration in the rat SI vibrissa cortex. *Journal of Neurophysiology* **54**, 615–635.

SIMONS, D. J. & CARVELL, G. E. (1989). Thalamocortical response transformation in the rat vibrissa/barrel system. *Journal of Neurophysiology* **61**, 311–330.

SIMONS, D. J. & WOOLSEY, T. A. (1984). Morphology of Golgi–Cox-impregnated barrel neurons in rat SmI cortex. *Journal of Comparative Neurology* **230**, 119–132.

STEFFEN, H. & VAN DER LOOS, H. (1980). Early lesions of mouse vibrissal follicles: their influence on dendrite orientation in the cortical barrelfield. *Experimental Brain Research* **40**, 419–431.

SUGITANI, M., YANO, J., SUGAI, T. & OOYAMA, H. (1990). Somatotopic organization and columnar structure of vibrissa representation in the rat ventrobasal complex. *Experimental Brain Research* **81**, 346–352.

VAN DER LOOS, H. & WOOLSEY, T. A,. (1973). Somatosensory cortex: structural alterations following early injury to sense organs. *Science* **179**, 395–398.

WAITE, P. M. E. (1973). Somatotopic organization of vibrissal responses in the ventro-basal complex of the rat thalamus. *Journal of Physiology* **228**, 527–540.

WHITE, E. L. (1979). Thalamocortical synaptic relations: a review with emphasis on the projections of specific thalamic nuclei to the primary sensory areas of the neocortex. *Brain Research Reviews* **1**, 275–311.

WONG-RILEY, M. (1979). Changes in the visual system of monocularly sutured or enucleated cats demonstrable with cytochrome oxidase histochemistry. *Brain Research* **171**, 11–28.

WOOLSEY, T. A., DIERKER, M. L. & WANN, D. F. (1975). Mouse SmI cortex: qualitative and quantitative classification of Golgi-impregnated barrel neurons. *Proceedings of the National Academy of Sciences of the USA* **72**, 2165–2169.

WOOLSEY, T. A. & VAN DER LOOS, H. (1970). The structural organization of layer IV in the somatosensory region (SI) of the mouse cerebral cortex: the description of a cortical field composed of discrete cytoarchitectonic units. *Brain Research* **17**, 205–242.