γ-Aminobutyric acid (GABA) immunoreactivity in mouse and rat first somatosensory (SI) cortex: description and comparison

Jolanta Chmielowska 2, Michael G. Stewart 1 and Rachel C. Bourne 1

1 Brain Research Group, Open University, Milton Keynes (U. K.) and 2 Nencki Institute of Experimental Biology, Warsaw (Poland)

(Accepted 7 July 1987)

Key words: Mouse; Rat; Somatosensory cortex; Barrel; Vibrissa; Anti-γ-aminobutyric acid (anti-GABA) serum; Immunocytochemistry

The location and morphological characteristics of γ-aminobutyric acid (GABA)-immunopositive cells and their processes were studied in rat and mouse first somatosensory (SI) cortex (including ‘barrels’) in layer IV, and layers above (I–III), and below (V and VI). In coronal sections of SI cortex of both species GABA-immunopositive cells and punctate profiles were found in each of layers I–VI. The cells were of various sizes; the largest, located in layers III and V of each species, resemble the large basket cells seen in Golgi-impregnated material. Most of the immunopositive cells were multipolar and circular or ellipsoidal in shape, but occasionally bipolar cells with fusiform perikarya were also seen. In coronal sections, immunopositive cells did not form a characteristic pattern. GABA-immunopositive cells were observed to be most numerous in the supragranular layers whereas GABA-positive punctate profiles were more numerous in layer IV. In tangential sections from layer IV of SI cortex of both species, GABA-immunopositive cells, processes and punctate profiles were visible throughout the entire barrel field. The pattern of distribution of immunopositive cells was similar (a) in two different morphological groups — i.e. the posteromedial barrel subfield (PMBSF) and the anterolateral barrel subfield (ALBSF) in rat barrel field, and (b) in PMBSF barrels of both rat and mouse (excluding differences due to structural dissimilarities between rat and mouse barrels). GABA-immunopositive neurons were grouped mainly in the barrel side and septum and were visible frequently in small clusters. In barrels of both species GABA-immunopositive cells were of a variety of sizes and ranged in shape from ellipsoidal to circular.

INTRODUCTION

Layer IV of the first somatosensory cortex (SI) of mouse and rat contains multicellular units termed ‘barrels’90. In the barrel field (i.e. the area occupied by barrels) the posteromedial barrel subfield (PMBSF) has large barrels arranged in 5 rows reflecting the arrangement of the largest vibrissae in 5 rows on the face of both species53,60. Physiological studies confirm that these large vibrissae, which are involved in whisking behaviour24, are represented by the large barrels in the PMBSF51,52. The anterolateral barrel subfield (ALBSF) has smaller barrels which do not show the same characteristic arrangement as the large barrels and they represent the smaller vibrissae of the snout, buccal pad and lip51,52. The homologous barrels in PMBSF of mice and rats have a different cytoarchitectonic structure54,61. In the mouse the barrels are composed of a ring of cells, the side, which surrounds a less cellular hollow, whereas in the rat the hollows are poorly defined with the distribution of cells being much more even between the sides and hollows. However, in the ALBSF of both species the structure of the barrels is similar to that of the PMBSF of the mouse.

Studies on the functional organization of the vibrissal representation in the SI cortex of mice and rats reveal many similarities. Electrophysiological52,53 and autoradiographic investigations using 2-deoxy-d-glucose (2-DG)5,10,11,16,29 have demonstrated the columnar organization of the vibrissal representation in SI cortex of both species. Studies of 2-DG metabolism have shown that stimulation of a single mystacial vibrissa of rat or mouse activates a single functional
column which extends through each of layers I–VI of the cortex and, at the level of layer IV, includes the appropriate barrel5,29. Despite the anatomical differences between the barrels of both species, the pattern of metabolic activity in the barrels is similar following stimulation of vibrissa. In mouse5 and rat29 the hollows are the regions of the barrel most activated. Electrophysiological studies42,43 have revealed similar properties for mouse and rat columnar units.

Considerable interest exists in the nature of inhibitory mechanisms, within both the barrels and the functional columns of which they are a part. γ-Aminobutyric acid (GABA) is the major inhibitory transmitter in the nervous system of mammals8,30,41. GABAergic neurons have most commonly been visualized in the mammalian cortex using antisera raised against glutamic acid decarboxylase (GAD), the enzyme of GABA synthesis37, or against the GABA molecule itself49. The existence of GABAergic neurons in SI cortex of mice and rats was first shown by Otterson and Storm-Mathisen59. A more detailed description of the distribution of GABAergic neurons in mouse and rat barrel field in layer IV of SI cortex has been provided from this6,7 and another laboratory4. In both species GABAergic cells were shown to be present throughout PMBSF barrels on tangential sections from layer IV but were most prominent in the barrel side and septa.

The purpose of the present study was: firstly, to describe the location and morphological characteristics of GABA-immunopositive cells and their processes in coronal sections of mouse and rat first somatosensory cortex including ‘barrels’ in layer IV, and layers above (I–III) and below (V and VI). Secondly, we proposed to determine whether the two different morphological barrel groups in rat barrel field (PMBSF and ALBSF) have a similar or different distribution of GABAergic neurons and processes and if, in view of the different cytoarchitectonic structure of PMBSF barrels in rats and mice, the GABA immunoreactivity varies in its distribution in barrels of these two species. A preliminary report of some of these data has already been published7.

MATERIALS AND METHODS

Six male and female mice and 7 male and female rats all approx. two months old and weighing 30 g (mice) and 250 g (rats) were used in the immunocytochemical experiments. Animals were perfused intracardially under ether anaesthesia, initially with 30 ml of Ca²⁺-free tyrode solution at 20 °C (pH 7.0) and subsequently with 50 ml (mice) or 150 ml (rats) of a fixative solution at 20 °C containing 0.1% glutaraldehyde and 2.5% paraformaldehyde in 0.1 M sodium phosphate buffer (PB) (pH 7.2–7.4) containing 0.002% CaCl₂. Brains were then removed and placed in cold (4 °C) fixative, as above, but minus glutaraldehyde. Vibratome sections of approximately 60 μm were cut either tangentially or coronally to the barrel field. From the 6 mouse brains 4 hemispheres were sectioned coronally and 8 tangentially whilst from the 7 rat brains 5 hemispheres were sectioned coronally and 9 tangentially. Vibratome sections were collected in 0.01 M phosphate-buffered saline (PBS) (pH 7.2–7.4). In some cases, to improve penetration of the GABA antibody, sections were placed in 0.01 M PBS containing 0.25% Triton X-100 for approx. 20 min prior to the beginning of the immunocytochemical reaction and in these cases a similar concentration of Triton X-100 was included in the media at the stages of the reaction involving the non-immune serum and the primary antiserum. Otherwise all sections were incubated similarly at 4 °C for pre-embedding GABA immunocytochemistry as described previously22,49.

Briefly the details are as follows. Sections were placed in the following media: 20% normal goat serum (NGS) (Ria, U.K.) for 0.5 h, anti-GABA serum (GABA code No. 7 — Hodgson et al.22) 1:1000 for 24–36 h, goat anti-rabbit (Miles Yeda) diluted 1:100 for 3 h. All sera were diluted with 0.01 M PBS and between each step the sections were washed in 0.01 M PBS. After a brief rinse in 0.05 M Tris-HCl buffer, pH 7.5 (T-HCl B) the tissue was pre-incubated in the same buffer containing 0.05% 3,3-diaminobenzidine tetrahydrochloride (DAB-grade II, Sigma). The immunocomplex was visualized by incubating the sections in 0.05 M T-HCl B containing 0.05% DAB with 0.01% H₂O₂ for 3–6 min at room temperature. The reaction was terminated by washing the sections in 0.05 M T-HCl B followed by a 15-min wash in 0.01 M PB. Sections were osmicated in 1.0% OsO₄ in PBS for approx. 60 min and then washed in 0.1 M PB prior to dehydration via alcohol and flat embedding in durepkan resin.
To serve as controls, tissue sections were processed using an immunocytochemical protocol identical to that described above with the exception that (1) the anti-GABA serum was replaced by NGS at an appropriate dilution in 0.01 M PBS or (2) the anti-GABA serum was replaced with normal rabbit serum. In each case the tissue for the control was either a coronal or tangential rat section. These showed no specific immunoreactivity associated with neuronal structures as opposed to the specific GABA-like immunoreactivity (GABA-LI), or immunopositive staining, observed in association with neuronal structures when the anti-GABA serum was present. In addition, 80-μm sections of cerebellar vermis were included in both control and GABA test experiments. Because GABA immunostaining of cerebellum has been well characterized with the GABA antiserum used in the present experiments it was possible to use the GABA immunoreactivity of the cerebellum as an index of the efficacy both of each GABA immunoreaction in mouse and rat SI cortex, and its specificity.

One mouse and one rat were intracardially perfused for light microscope histology and sections, cut coronal and tangential to the barrel fields, were Nissl-stained. Photographs were taken on either a Zeiss Photomicroscope III or a Reichert Polyvar Microscope at magnifications ranging from ×12.5 to ×1000. A graticule scale (in 0.01 mm units) was used to accurately calibrate the magnification of the microscopes. Estimations of cell size (measured as maximum diameter — $D_{\text{max}}$) of immunopositive and immunonegative profiles, were made on the digitizing tablet of a microcomputer measuring system (Imagan, Leitz, U.K.), either from photomicrographs or directly from the microscope slides via a camera lucida projection arm. Values, where given, are the means of estimations on up to 100 cells but are intended only as approximations of cell sizes and should not be taken as fully quantitative measures.

RESULTS

Morphology of Nissl-stained mouse and rat barrel fields

The appearance of the mouse and rat barrel fields shown here by Nissl staining (Fig. 1) is similar to that demonstrated by Welker and Woolsey in a study which compared barrel structure in layer IV of mouse with that of rat first somatosensory cortex (SI). Fig. 1A shows that all barrels in mouse barrel field — both the large barrels arranged in 5 rows in the PMBSF and the smaller barrels in the ALBSF — have basically a similar morphology, i.e. a distinct side composed of a ring of cells and a less cellular hollow. Fig. 1B shows that two distinct morphological groups of barrels are evident in rat barrel field. The large barrels arranged in 5 rows in the PMBSF have poorly defined hollows and sides with the distribution of cells being much more even between the sides and hollows than in the mouse PMBSF and ALBSF, whereas ALBSF barrels (of the rat) have a structure which is very similar to that of mouse barrels. Simons and Woolsey and Welker and Woolsey have also described several other morphological differences between mouse and rat barrels: the size of barrels — larger in rat; the shape of barrels — ellipsoidal in mouse and round in rat, and the size of barrel cells — larger in rat than mouse.

GABA immunoreactivity in mouse and rat barrel field

GABA immunoreactivity of almost the entire mouse and rat barrel fields (PMBSF and ALBSF) is shown at low magnification in Figs. 2A and B. At this magnification the appearance of barrels after GABA immunostaining reflects the cytoarchitectonic pattern revealed by Nissl staining and shows, similarly, the differing appearance of rat and mouse PMBSF barrels (see Discussion). However, at such a low magnification the distribution of GABA-immunopositive and immunonegative cells cannot be seen clearly.

Figs. 3B, 4B, 5B and 6B show at higher magnification the distribution of GABA-immunopositive cells and processes, and GABA-immunonegative cells, over mouse and rat PMBSF barrels, and mouse and rat ALBSF. Immunopositive cells are visible over the entire barrel components (sides, hollows and septa) but are not distributed uniformly. Whilst they are frequently visible in small clusters they can also be found alone. It is not possible to define precisely the boundaries of the barrel wall in the immunostained sections viewed at high magnification, but there appears to be a similar pattern of distribution of GABA immunoreactivity in the PMBSF and ALBSF of both species: most notably, GABA-immunopositive cells
are grouped mainly in the barrel side and septa.

Figs. 3C,D,E; 4C,D,E; 5C,D,E and 6C show GABA-immunopositive cells taken from different parts of mouse and rat barrels including the side, hollow and septum. Immunopositive cells range in shape from circular to ellipsoidal. The mean maximum diameter was 18 μm for mouse PMBSF barrels, 16 μm for rat PMBSF barrels, 15 μm for mouse ALBSF barrels and 20 μm for rat ALBSF barrels. Processes of immunopositive cells are visible also in Figs. 3–6. Some of these processes are orientated towards the barrel hollows whilst others are orientated towards the wall. Immunopositive processes radiate from the soma of some cells in all directions. The shallow depth of the immunoreaction zone (<5 μm) did not, however, permit the investigation of entire cells and their processes. Small immunopositive punctate profiles are also visible in Figs. 3, 4, 5C–E and 6C. These

Fig. 1. A: mouse left hemisphere. B: rat left hemisphere. Tangential (50 μm) vibratome sections Nissl-stained from layer IV of SI cortex. Orientation markers: p, posterior; a, anterior; m, medial; l, lateral. PMBSF, posteromedial barrel subfield with 5 rows of barrels A–E; ALBSF, anterolateral barrel subfield. See text for comment on barrel structure. Bars = 500 μm.

Fig. 2. A: mouse right hemisphere. B: rat right hemisphere. Tangential (50 μm) vibratome sections from layer IV GABA-immunoreacted; orientation markers as in Fig. 1. Bars = 500 μm.

Fig. 3. A: 5 rows of barrels (rows A–E) in the mouse PMBSF in layer IV of a GABA-immunoreacted tangential section. Bar = 500 μm. B: higher-magnification micrograph of area outlined in A; the outlined areas in B are from different parts of the barrels and contain the GABA-immunopositive cells shown at higher magnification in C–E. The cells vary in shape and size. Bar = 100 μm. C: cells from the side/septum. D,E: cells from hollows; orientation markers as in Fig. 1. Bars for C–E = 25 μm.

Fig. 4. A: 3 rows of barrels (rows C–E) in the rat PMBSF in layer IV in GABA-immunoreacted tangential section. Bar = 500 μm. B: higher-magnification photo-montage of A. Solid arrows indicate the barrels and open arrows indicate the septum between the rows of barrels. Bar = 160 μm. The outlined areas from different barrels contain the GABA-immunopositive cells shown at higher magnification in C–E. These cells are of varying size and range in shape from ellipsoidal to circular. Small arrows indicate cell processes. Bars for C–E = 25 μm. Orientation markers as in Fig. 1.
are confined more to the barrel hollow than the side. In control (not shown) tangential sections of rat and mouse barrel cortex (with NGS substituted for the GABA antiserum) it is clear that no specific GABA-like immunoreactivity is associated with neuronal structures in either species. A similar result was obtained in the control when normal rabbit serum was substituted for the GABA antiserum. GABA immunoreactivity in the cerebellum of both mouse and rat appeared identical to that shown in previous experiments with the same antiserum and the cerebellar controls, in which NGS was substituted for the GABA antiserum, were devoid of specific GABA immunoreactivity.

**Appearance of GABA immunoreactivity of mouse and rat first somatosensory cortex in coronal sections**

GABA-immunopositive cells are visible in each layer (I–VI) of the somatosensory cortex of both species (Fig. 7, mouse and Fig. 8, rat). There is no characteristic arrangement of immunopositive cells visible either in barrel layer IV or in layers above (I–III), and below (V and VI). Immunopositive cells are sometimes visible in groups but are also found alone (Figs. 7B and 8B). GABA-positive cells were observed to be most numerous in the supragranular layers. In every layer (I–VI) of rat and mouse cortex immunopositive cells of different shape can be seen: circular, ellipsoidal and fusiform but characteristically in layer IV of both species most immunopositive cells were circular in appearance (Figs. 7G and 8F). Many cells were multipolar with circular or elongated cell bodies (Fig. 7E,F and 8C,D,G). The orientation of dendritic processes of immunopositive cells was markedly different. In layers II, III and V the very large immunopositive cells shown in Fig. 7 have their primary dendrites orientated perpendicular to the pia and white matter. The secondary dendrites of the cell shown in Fig. 8C are orientated in the same direction as the primary dendrites. Similarly, the proximal parts of primary dendrites from a number of other cells show the same orientation.

Some cells have dendrites orientated in two directions: horizontally directed shafts arise from the middle region of the cell body whilst vertically orientated dendrites arise from the top and bottom of the cell body (Fig. 8C). In each of the cortical layers (I–VI) immunopositive cells of different size can be seen but, characteristically for both species, cells with very large perikarya (up to 30 μm) are found at two levels: layers II/III and V (Figs. 7E and 8C,G). These large multipolar cells resemble the large Golgi-impregnated neurons occurring in layers III and V of different cortical areas. Apart from these large cells neurons of medium and small size were visible. GABA-immunopositive punctate profiles were observed in all layers of the somatosensory cortex of both species, but with a higher density in layer IV.

**DISCUSSION**

The analysis of the distribution and morphological appearance of GABAergic inhibitory neurons forms a basis for investigation of the functional inhibitory mechanisms that exist in somatosensory cortex. In the present study we have concentrated on two main points: firstly, the appearance over the layers of the mouse and rat SI cortex of GABAergic cells that seem to be of interest in relation to the functional columnar organization of this region of the cortex. Secondly, the appearance of GABAergic cells over the unusual cytoarchitectonic units, i.e. barrels, which are the anatomical parts of the functional columns.

**GABA immunoreactivity in mouse and rat barrel field**

The data obtained in the present study have shown that the pattern of distribution of GABA-immunopositive...
itive cells is very similar in (1) two different morphological barrel groups — ALBSF and PMBSF — and (2) in PMBSF barrels of mouse and rat (apart from the different cytoarchitectonic structure of these barrels in the two species). In all barrels of both species the GABA-immunopositive cells were present over the entire barrel elements, although they were grouped largely in the barrel periphery (the 'side' in mouse) and in the septum, which is in agreement with our previous findings. These data agree also with that on the localization of the GABA-synthesising enzyme — GAD immunoreactivity — in barrel cortex of the rat and mouse. However, our data show more GABA-immunoreactive neurons both in the septa between the barrels in each row, and between the rows of barrels, than were shown in the study by Lin et al. which reported very few GAD-immunopositive neurons and terminals in the septal regions. There are two possible explanations for these discrepancies, one of which may have a basis in the findings of a study by Somogyi et al. In this a comparison was made of the density and distribution of neuronal elements in the visual cortex of the cat. immunoreactive for GABA and GAD (without pretreatment with colchicine as in the work of Lin et al.). and fewer GAD-, compared to GABA-, positive cells were found. A second explanation may be connected with the difference in the width of the septa between the barrels and rows of barrels shown in the paper by Lin et al., and that observed in the present case. This is between 10 and 20 μm in Fig. 1 showing PMBSF of rat in Lin et al.'s paper, whereas we find this width in PMBSF of rat to be between 30 and 80 μm, and in two previously published neuroanatomical papers this can be estimated as between 25 and 65 μm in Figs. 1 and 6 of Simons and Woolsey and between 40 and 80 μm in Fig. 5 of Welker and Woolsey. However, these differences not withstanding, there is good agreement between the anti-GABA and anti-GAD investigations as to the identity of GABA-ergic components in the rodent barrel field.

The morphology and size of GABA-immunopositive cells presented here, and of GAD-immunopositive cells reveal many similarities. Both GABA- and GAD-positive cells are multipolar with smooth dendrites. They demonstrate the features of the class II neurons shown in Golgi preparations of mouse and rat barrel cortex and some to a group of local plexus neurons visible in mouse and rat barrel cortex, and one type II cells the GABAergic cells belong, can only be provided by combined Golgi- and GABA-immunochemical techniques.

Rat PMBSF and ALBSF barrels differ in cytoarchitectonic structure: rat ALBSF barrels resemble those in mouse ALBSF in possession of sides and hollows. The present study demonstrates a similar pattern of distribution of GABAergic cells in both groups of rat barrels. The small ALBSF barrels which represent the sinus hair of the snout, buccal pad and lip, have not been analysed in the literature in as much detail as the PMBSF barrels which represent the large mystacial vibrissae involved in 'whisk-
ing behaviour'. However, there are some data that indicate the existence of physiological similarities between these two different morphological groups. Histochemical studies have demonstrated a similar pattern of cytochrome oxidase (CO) and succinic dehydrogenase (SDH) activity, with the highest value in the barrel centre in ALBSF and PMBSF barrels of the rat.

It is known that PMBSF barrels of mice and rats differ markedly in cytoarchitecture. The ratio of the density of the cell bodies in the hollows:side is much higher in rat than in mouse PMBSF barrels where the side:hollow ratio is 1.6 (refs. 40, 60). Apart from these cytoarchitectonic differences the distribution of GABA-immunopositive cells in PMBSF barrels of both species is very similar to that of GAD immunoreactivity in barrel cortex of rat and mouse. Other similarities, additional to those of function and anatomy, have been established between the PMBSF barrels of both species. The data for 2-DG and histochemical studies show a similar pattern of activity in mouse and rat PMBSF barrels. The densest 2-DG labelling and highest CO activity were found in the barrel centre. The properties of barrel units such as the directional selectivity, types of bioelectric waveforms and whisker configurations studied electrophysiologically in mouse and rat PMBSF barrel cortex, were very similar. Also, anatomical studies provide data indicating similarities (between rat and mouse PMBSF barrels) in the morphology of barrel cells, the pattern of their axon and dendritic distribution, and the concentration of termination of thalamocortical fibres in the barrel centres. On the basis both of the present data, and those cited above on the distribution of GABAergic elements in the barrel cortex, it may be proposed that in barrels of rats and mice (in both ALBSF and PMBSF barrels) there are similarities not only in the transmission and transformation processes of the vibrissal system, but also in the case of functionally inhibitory mechanisms.

**Distribution of GABA immunoreactivity in mouse and rat first somatosensory cortex**

The present study has concentrated on the part of the rodent's somatosensory cortex which contains the 'barrel region' of layer IV, and the layers above (I, II and III) and below (V and VI). GABA-immunopositive cells were visible in this part of the cortex over all cortical layers (I–VI) in agreement with the data of Ottersen and Storm-Mathisen on the same species, and also with that in monkey sensorimotor cortex and other cortical areas such as striate cortex of kittens where GABAergic elements were investigated using either antisera against GAD or by injection and uptake of [3H]GABA. Here, in coronal sections of mouse and rat SI cortex, no distinct pattern of GABA-immunopositive cells could be distinguished in barrel cortex in layer IV, and in layers above and below. Although a fully quantitative analysis of the distribution of GABA-immunoreactive neurons has not been carried out here, they appear to be most numerous in the supragranular layers of the SI of both species. In other species, e.g. monkey sensorimotor cortex or cat visual cortex, a similar high number of GABA- and GAD-positive neurons were noted in the supragranular layers, and also in layer VI. Clearly a detailed quantitative study is required to determine the proportions of GABAergic neurons in the SI cortex of rodents.

The distribution of other GABA-immunopositive elements was also observed here. GABA-immunopositive punctate profiles were visible in all layers of mouse and rat somatosensory cortex with an apparent higher density in layer IV ('barrel layer'). Ottersen and Storm-Mathisen noted in the same species a slightly lower density of GAD-immunopositive punctata in layer V. This pattern of distribution of GABAergic punctate profiles reflects the pattern of CO activity in rat somatosensory cortex shown recently by Land and Simons. They observed radially orientated zones of CO activity in which layer IV is characterised by the highest level and layer Va is relatively low in CO staining in comparison to the rest of the layers. Striking laminar similarity between GABAergic punctate profiles and CO staining has been shown in monkey striate cortex.

The GABA-immunopositive cells shown in the present study in coronal sections differ in size and shape throughout the layers of mouse and rat somatosensory cortex. None of the immunoreactive neurons exhibit characteristics of pyramidal cells. In layers III and V of both mouse and rat cortex we found GABA-immunopositive cells with soma size characteristically around 30 µm and of multipolar form. These GABA-immunopositive neurons resemble
closely the large Golgi-impregnated basket cells described in the human cortex and also in mouse, rat and monkey cortex. The Golgi-impregnated basket cells had large somata (20–30 μm) and their axons terminated in characteristic ‘pericellular nests’ around the somata of pyramidal cells. Neurons with similar features but without the characteristic pattern of axon termination were found to be immunoreactive for GAD and to take up [3H]GABA in monkey sensorimotor cortex, and were also demonstrated in cat visual cortex by the combined technique of Golgi-gold toning and GAD immunostaining. The large GABA-immunopositive neurons demonstrated here were located mainly in layers III and V, a finding which is consistent with the appearance of large GAD-immunopositive cells and Golgi-impregnated large basket cells in the same layers in monkey somatosensory cortex.

Other GABA-immunopositive cells visualized in the present study were of small to medium size, predominantly multipolar and circular in shape but occasionally bipolar with elongated fusiform perikarya. Many neurons had their primary and secondary dendrites orientated perpendicular to the pia and white matter but cells with their primary dendrites in the horizontal plane were also encountered. The diversity of somal shape, size, laminar location and variety of dendritic patterns suggests that GABAergic neurons in mouse and rat first somatosensory belong to several different morphological classes of intrinsic neurons. On the basis of a comparison of the morphology of GAD-immunopositive cells or cells taking up [3H]GABA and the morphology of Golgi-impregnated neurons it was suggested that there are several GABAergic cell types: small basket cells, neurogliaform cells, local plexus neurons, chandelier cells, and bouquet or bitufted neurons. Some of these were identified in Golgi-impregnated sections from mouse and rat barrel cortex in layer IV, i.e. neurogliaform cells, local plexus neurons, chandelier cells, and bouquet or bitufted neurons. These cells were also found in the barrel cortex of monkey somatosensory cortex.

Possible functional implications

The results obtained in the present study and in previous work on the distribution of GABAergic cells in mouse and rat barrel field allow us to make some provisional suggestions about the role of inhibitory neurons in neuronal circuitry in barrel cortex. On tangential sections from layer IV of both mouse and rat the GABAergic cells were visible over entire barrels although they were grouped mainly in the barrel side and septum. The morphological diversity of barrel GABA-immunopositive cells suggests that they may belong to different subgroups of class II smooth neurons. We may postulate that the inhibitory neurons located in the barrel centre (i.e. hollow) have different functions than those of cells located in the barrel periphery and septum. The hollow is known as a focus of dense thalamocortical axonal terminals which transmit information arising from lower levels of the ascending vibrissal-barrel system. Anatomical studies have shown that some non-spiny multipolar cells located in hollows form a high proportion of their synapses with thalamocortical terminals. The GABAergic inhibitory neurons located in the barrel hollow could play a role in preservation of specific information from a single whisker. Electrophysiological data have shown that most units recorded in the barrel centre respond only to stimulation of one whisker whilst units recorded in the barrel periphery respond to deflection of several whiskers. Inhibitory neurons located in the barrel centre could also maintain specific features of peripheral stimulus such as the direction, velocity and amplitude of vibrissal deflection.

Inhibitory neurons located in the barrel side and septa could participate in the functional interactions that occur between adjacent barrels as has recently been demonstrated electrophysiologically. In rat vibrissal cortex Simons has observed the presence of response suppression of barrel units which are dependent on factors such as the angular direction in which whiskers are moved, the sequence in which they are deflected and the numbers of whiskers involved. He comments that a preceding deflection of B1 vibrissa inhibits the unit’s response to subsequent deflections of B2 in a time-dependent fashion, or that a preceding displacement of C3 substantially reduced the cell’s response to subsequent displacement of C3 at interdeflection intervals of 10, 20 and 100 ms. Anatomical data may provide a basis to support the idea of interactions between the barrels. The neurons of a single barrel are known to distribute their dendritic processes to adjacent barrels in both mouse and rat. Class II cells (presumed inhibitory neurons) are also known to send their processes...
deep into adjacent barrels. In the present study, GABAergic cells have also been found between the barrels. In Golgi preparations, the cells from the septa were observed to distribute their dendrites to several barrels. The GABAergic neurons located in the septa could be involved in functional interactions between the barrels. They could also be involved in inhibitory interactions with the terminations of the small callosal projections which were found recently in the septa between the barrels.

GABA-immunopositive cells were visible in every layer of mouse and rat SI cortex. They vary in size and shape, suggesting morphological diversity, and as a consequence of this they could play different functions in cortical information processing. Recent studies, however, both electrophysiological and using 2-DG, demonstrate that a single vibrissa is represented in the cortex in the form of a single functional column with definable shape and size. The question which arises is: what is the anatomical basis of the mechanism which inhibits the horizontal spread of information and can determine the size and shape of a single functional vibrissal column? Among the inhibitory neurons 'the large basket cells characterised by numerous horizontally disposed axon collaterals extending for 1–2 mm in the antero-posterior direction' are suggested to 'provide suppression of activity of large fields of surrounding neurons and to provide the simultaneous inhibition of cells in neighbouring vertical columns'. Indeed large GABA-immunopositive cells, resembling the large basket cells in e.g. cat, were found in mouse and rat SI cortex in the present study. Other presumed inhibitory cells like local plexus or neurogliaform cells are suggested to 'exert inhibitory influence in much more restricted fields that could include numerous small neurons'. They could take part in inhibitory mechanisms existing inside a single vibrissal column. For example, the anatomical and electrophysiological data might support the hypothesis that in rodents a single vibrissal column contains microcolumns of neurons which contain only one subtype receptor.

ACKNOWLEDGEMENTS

This work was supported by grants to M.G.S. and J.Ch. from the Open University Research Committee, The Polish Academy of Sciences and The British Council. Grateful thanks are expressed to members of the Open University Brain Research Group and to Dr. M. Kossut of the Nencki Institute for helpful comments on the manuscript. The GABA antiserum (code GABA-72) was a gift from Dr. J. Somogyi of Semmelweis University Medical School, Budapest, Hungary.

REFERENCES

14 Gabott, P.A. and Somogyi, P., Quantitative distribu-
51 Welker, C., Microelectrode delineation of fine grain soma-
56 White, E.L., Thalamocortical synaptic relations: a review with emphasis on the projection of specific thalamic nuclei to the primary sensory areas of the neocortex. *Brain Res. Rev.*, 1 (1979) 275–311.