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SYNAPTIC RELATIONSHIPS OF A TYPE OF GABA-IMMUNOREACTIVE NEURON (CLUTCH CELL), SPINY STELLATE CELLS AND LATERAL GENICULATE NUCLEUS AFFERENTS IN LAYER IVC OF THE MONKEY STRIATE CORTEX

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Abstract—The precise stimulus specificity of striate cortical neurons is strongly influenced by processes involving gamma-aminobutyric acid (GABA). In the visual cortex of the monkey most afferents from the lateral geniculate nucleus terminate in layer IVC. We identified a type of smooth dendritic neuron (clutch cell) that was immunoreactive for GABA, and whose Golgi-impregnated dendrites and axon were largely restricted to layer IVCβ. The slightly ovoid somata were 8–12 μm by 12–15 μm and the dendritic field was often elongated, extending 80–200 μm in one dimension. The axonal field was 100–150 μm in diameter and densely packed with large bulbous boutons. Although mainly located in IVCβ both the dendritic and axonal processes entered IVCα.

Fine structural features of GABA-immunoreactive and-impregnated clutch cells and impregnated spiny stellate cells were compared. Clutch cells had more cytoplasm, more densely packed mitochondria and endoplasmic reticulum, and made type II as opposed to type I synapses. A random sample of 159 elements postsynaptic to three clutch cells showed that they mainly terminated on dendritic shafts (43.8–58.5%) and spines (20.8–46.3%), rather than somata (10–17%). The majority of the postsynaptic targets were characteristic of spiny stellate cells. This was directly demonstrated by studying synaptic contacts between an identified GABA positive clutch cell and the dendrites and soma of an identified spiny stellate cell.

The termination of clutch cells mainly on dendrites and spines of spiny stellate cells suggests that they interact with other inputs to the same cells. Following an electrolytic lesion in the ipsilateral lateral geniculate nucleus we examined the distribution of degenerating terminals on three identified spiny stellate neurons in layer IVCβ. Out of eight synapses from the lateral geniculate nucleus one was on a dendritic shaft, the rest on spines. Only a small fraction of all synapses on the cells were from degenerating boutons. A clutch cell within the area of dense terminal degeneration was not contacted by terminals from the lateral geniculate nucleus.

The results show that layer IVC in the monkey has a specialized GABAergic neuron that terminates on spiny stellate cells monosynaptically innervated from the lateral geniculate nucleus. Possible functions of clutch cells may include inhibitory gating of geniculate input to cortex; maintenance of the antagonistic subregions in the receptive fields; and the creation from single opponent of double colour opponent receptive fields.

In the primary visual cortex of monkeys, layer IV is the major recipient zone of the specific visual afferents from the lateral geniculate nucleus[5,19,24] (LGN). This is reflected in the response properties of the majority of cells in layer IVC, which are monocularly driven and have small receptive fields, many of them non-oriented like those of cells in the LGN and unlike cells in all other layers.[4,17,23,26] Within layer IV axons from the parvocellular geniculate layers terminate in sublayers IVA and IVCβ whereas axons from the magnocellular geniculate layers terminate in layer IVCα.[5,19,24] The contrasting properties of magno- and parvocellular LGN afferents are reflected in the sublayers of IVC since neurons in IVCα have lower thresholds to luminance contrast[4,17] whereas the responses of some cells in IVCβ are colour opponent.[4,23,36,46] However, the step from LGN axons to layer IV cortical neurons is not a simple relay. Firstly, new properties such as orientation selectivity[6,17,36,49] and double colour opponent fields[46] emerge. Secondly, there are at least 30 times as many cortical cells in the LGN recipient layers than in the LGN,[48] implying a large divergence if all the cells are innervated by LGN terminals. Such divergence is

Abbreviations: GABA, gamma-aminobutyric acid; GAD, glutamate decarboxylase; HRP, horseradish peroxidase; LGN, lateral geniculate nucleus.

strongly suggested by the lateral spread of the axons in layer IV.[5] As 20–40 parvocellular LGN axons may overlap at any one point in layer IVCβ,[4] there could also be extensive convergence onto cortical neurons. Notwithstanding the divergence and convergence of individual axons, the retinotopic map in layer IV is very precise.[4,25,26]

It has been suggested that the retinotopic precision and narrow tuning curves of cells in layer IV[4] and in other layers and areas[1,2] are brought about by local processing among the cortical neurons. The neuronal composition of layer IV is relatively well known. As in other primary sensory areas this layer is populated by small, densely packed cells. The best known types are the spiny stellate cells (for review see Ref. 38), but several varieties of smooth dendritic cells have also been described.[44,70,72]

In contrast, little is known about the synaptic relationship among the cells and between them and the LGN afferents. The position of putative inhibitory interneurons in the synaptic circuit could be particularly important because in other species and in other parts of the visual pathway they enhance the precision and specificity of responses[62] (see Ref. 57). It is therefore of interest that layer IVC contains a small smooth dendritic cell whose varicose dense axon is largely confined to layer IVC. This cell has variously been called "spider cell",[52] "clewed cell",[72] "midget category II cell",[70] "smooth dendritic stellate neurons with beaded axons".[37,40,44] The axon establishes type II or symmetrical synapses,[44] and by analogy with other smooth dendritic cells that form type II synapses, these cells may be GABAergic. Separate immunocytochemical studies indeed demonstrate that both GABA[30] and its synthesizing enzyme glutamate decarboxylase (GAD)[18] are present in layer IVC. However, until recently it was impossible to determine whether the GABAergic markers were present in cells specifically associated with layer IV or in cells that distributed their processes in a random fashion amongst the layers. We recently introduced a method that reveals the processes of the neuron by Golgi impregnation, the method that had originally led to the discovery of these cells, and also allows the demonstration of GABA in the same cell.[67] The aim of the present study was to establish whether this particular neuronal type, seemingly associated with the LGN recipient layers, contained GABA and could therefore play a role in inhibitory interactions. We also tried to establish their synaptic relationships to spiny stellate cells and to LGN afferents.

Because of their association with the sublayers innervated from the LGN, it has long been assumed that spiny stellate cells are the major recipients of the visual afferents (for reviews see Refs 38 and 54). In other species this has been demonstrated directly,[12,22,50,64] but direct evidence is still lacking in the monkey. Therefore, to evaluate the termination pattern of the GABA-containing neurons on spiny stellate cells, we also sought evidence in a pilot study that spiny stellate neurons were indeed monosynaptically contacted by the terminals of LGN axons.

EXPERIMENTAL PROCEDURES

Animals

The neurons analysed were selected from three adult monkeys (one *Macaca mulatta*, two *Macaca fascicularis*) all seropositive for *Herpes simiae* and therefore unsuitable for long-term experiments. Shortly before perfusion, the two cynomolgus monkeys had received a microinjection of [^{3}H]GABA in a small area of the striate cortex for studying the uptake of the amino acid and the results have been reported elsewhere.[66] The neurons decribed in this paper were collected from an area well outside the injection sites and there is no reason to suppose that the injections affected their synaptic connections. In the rhesus monkey electrolytic lesions were made in the lateral geniculate nucleus (LGN) on one side. Briefly, the animal was sedated with ketamine hydrochloride, (Ketalar, Parke-Davis, 10 mg/kg, i.m.) then deeply anaesthetized with sodium thiopentone i.v. (Pentothal, May and Baker) and placed in a stereotaxic instrument. Skin was cut, bone removed and dura cut above the central sulcus on one side. A bipolar electrode consisting of two 0.25 mm stainless steel wires glued together, insulated except for their 1 mm tapered tips, and offset vertically by 1.5 mm, was lowered into the brain. Evoked potentials to a stroboscopic light were recorded from each tip and between the tips. When the lower tip was within the body of the LGN an electrolytic lesion was made by passing 5 mA for 25 s with the tip as cathode. This was done at 5 sites spaced 1 mm apart in a rostrocaudal line through the LGN from approximately Ant. 7.0 and Lat. 12.0 to Ant. 3.0 and Lat. 13.0. The animal was perfused 4 days later. This survival time was chosen on the basis of preliminary experiments in two other animals.

Tissue processing

For fixation all three monkeys were deeply anaesthetized with a lethal dose of Pentothal, then perfused transcardially with Tyrode's solution for 2 min followed by fixative containing either 0.5 or 2% paraformaldehyde (TAAB Laboratories, Reading, U.K.) mixed with either 1.25 or 2.5% glutaraldehyde (TAAB Laboratories, Reading, U.K.) in 0.1 M phosphate buffer solution (pH 7.4). The brains were then removed and kept in fixative overnight. A block of the thalamus containing the LGN was dissected, cut on a cryostat and stained with Cresyl Violet for histological verification of the lesion. The lesion was centred on the representation of the horizontal retinal meridian and destroyed about one quarter of each parvocellular layer throughout the rostrocaudal extent of the LGN. The dorsal magnocellular layer was also affected in the caudal part of the LGN. Small blocks were dissected from the occipital operculum and processed for rapid Golgi-impregnation.[66] Sections were cut at 80 μm and gold-toned[10] as decribed earlier.[14] Material obtained from the animal with geniculate damage was not processed for gold-toning since the experiment was performed before the introduction of this procedure. Neurons were drawn using a $\times 100$ oil immersion objective, photographed in the light microscope and reembedded for correlated light and electron microscopy. Layering of the cortex followed the scheme of Lund and Boothe.[39] Postsynaptic elements were analysed quantitatively from electron micrographs of three cells obtained from the two cynomolgus monkeys. Several hundred serial sections were cut of these cells and each bouton in the plane of the section, and identified as originating from a clutch cell, was followed until the postsynaptic element could be identified. Since the plane of the section was not adjusted to conform to the axonal arbor in any particular way, this sampling is considered random.

Postembedding GABA-immunocytochemistry

Semithin, 0.5 μm sections were cut from the somata of all but two Golgi-impregnated neurons examined in this study, and prepared for postembedding GABA-immunocytochemistry by using an antiserum directed against glutaraldehyde-conjugated GABA. The procedures for producing and characterizing the antiserum (code No. 9) have been described elsewhere.[21,68] Immunocytochemistry was carried out as reported previously[67] using the same reagents and the unlabelled antibody peroxidase–antiperioxidase method.[69] As a control, alternate sections of the same cells were incubated with the GABA antiserum that was preincubated with GABA coupled to polyacrylamide beads as described earlier.[21]

RESULTS

Clutch cells are easily recognized from their beaded, densely ramified axons, and smooth dendrites, and from their remarkable restriction to layer IVC. These features were used to select 11 neurons for drawing from about 50 similar cells that were found. The findings will be used to delineate these cells from other smooth dendritic neurons and to show that they are analogous to "clutch cells" in the striate cortex of cat[31] (see Discussion). For brevity the appellation is also used in the results section.

Position of clutch cells and the distribution of their processes

The somata of all 11 neurons (N_{1-11}) were located in layer IVCβ. Within this layer they may occupy any dorsoventral position. The somata were either round, or ovoid with long and short axes of 12–15 and 8–12 μm, respectively (Figs 2A, B, 3, 4, 5A–D and 9). The smooth dendrites arose from the perikaryon in groups from 2 to 3 main stems (Figs 2, 3 and 9). They had a typical wavy and sometimes reflexive course. As a result most dendrites terminated within 100 μm of the soma giving a dendritic field not greater than 100–200 μm at its widest, but smaller dendritic fields of only about 80–100 μm in lateral extent were common. Horizontal elongation was also found near the border with layer V (Figs 2A, B, 3 and 9). The dorsally oriented dendrites freely crossed into IVCα, but ventrally only a few dendritic tips entered layer V (Figs 2 and 3).

The most characteristic identification mark of the clutch cell was the profusely branched and crowded varicose axon peppered with large, bulbous boutons (Figs 1–3, 4A and 9). The axon initial segments originated at the pial pole of the soma, the axons then followed an ascending course for 50–100 μm before recurving ventrally. They gave off a few main collaterals which branched frequently to produce a very dense axonal plexus 100–150 μm in diameter. Almost all the axonal branches remained in the lower two thirds of layer IVC (Figs 2 and 3). Only occasional axon collaterals departed from layer IVC. For example, neuron N_2 in Fig. 2 had a collateral descending to layer VI, where it emitted a few short collaterals with similar boutons to those found in layer IVC. It should be noted that the longer collaterals are not always easy to follow with the Golgi method and some of these cells may have restricted projections to other layers as suggested by some earlier results, especially those obtained in young monkeys[37,40] and in the cat.[31] Some of the boutons of clutch cells were apposed to other somata, suggesting axosomatic synaptic contacts. However, the vast majority of the boutons were in the neuropil (Figs 1 and 4A).

GABA immunocytochemistry

Several types of smooth dendritic cells in the cortex have been shown to contain GABA[65,67] or GAD[11,63] and are presumably GABAergic. One purpose of the present study was to determine whether the clutch cells in the monkey cortex may also be GABAergic. Therefore, semithin sections were immunocytochemically reacted of all but two (N_7 and N_{11}) of the 11 neurons for GABA. Using the peroxidase–antiperoxidase method, the presence of the dark brown reaction endproduct showed that all the tested clutch cells were positive for GABA (e.g. Figs 4B, G, I and 5B, D). When the same antiserum was incubated with GABA attached to a solid phase carrier prior to application to the sections, no immunoreaction was observed (Fig. 4C, H), and only the thin rim of colloidal gold along the plasma membrane marked the identified clutch cells (Fig. 4C, H). Identified gold-toned spiny stellate cells (17 tested) never showed GABA immunoreactivity.

Comparison of the fine structural characteristics of clutch cells and spiny stellate cells

The two types of neuron specifically associated with layer IV are spiny stellate cells and clutch cells. As only the latter were immunoreactive for GABA we studied whether this biochemical difference was accompanied by fine structural differences and contrasting synaptic connections.

The somata of clutch cells were larger (12–15 μm longest extension) and more irregular in shape than those of neighbouring spiny stellate cells. Clutch cells had a higher cytoplasm to nucleus volume ratio (Figs 4D and 5A, C) than did nearby spiny stellate cells (Figs 6F and 7B). The nuclear membrane of both cells was usually smooth (Figs 5A, C and 7B), with only occasional small invaginations on clutch cell nuclei. This is in contrast with other cortical smooth dendritic neurons which have deep nuclear invaginations (for review see Ref. 51). The nuclei of spiny stellate cells were smaller and showed a more regular round profile in cross section (Figs 4 and 7B). A conspicuous difference between the two cell types was the much more plentiful mitochondria and rough endoplasmic reticulum in the cytoplasm of clutch cells (Figs 4D and 5A, C).

The synaptic input of the somata of both cell types was similar and consisted of 1–4 boutons in a single ultrathin section (Figs 5E, 7B, C and 8F). The classification of synaptic contacts on the somata of

clutch cells was made difficult by the gold precipitate along the plasma membrane. Several type II contacts could be identified but the possibility of the presence of type I contacts, reported earlier,[44] cannot be excluded (Fig. 5E). The input to the dendrites will be described later.

The most obvious fine structural difference between the two cell types was in the structure of synaptic contacts they made onto other neurons; spiny stellate cells established type I, or asymmetric synapses (Fig. 8B), clutch cells in contrast made type II, or symmetric contacts (e.g. Fig. 6). To elucidate the site of action of clutch cells we made a detailed study of their postsynaptic targets.

Postsynaptic targets of clutch cells

All parts of the axonal field of three clutch cells ($N_{6\,8}$) were sampled to obtain a random sample of postsynaptic targets. The synaptic boutons were traced in serial sections. Every bouton in the plane of section was followed until the synaptic contact was encountered. In this part of the study 130 boutons were analysed and they established an average of 1.22 synaptic contacts. Two boutons contacted three targets and 25 boutons contacted two. Thus 159 postsynaptic elements were encountered altogether in the random sample (Table 1). In addition a further 17 postsynaptic targets were identified during the course of serial sectioning aimed at particular boutons preselected from the light microscope (Table 1). In the random sample the targets were neuronal somata (10–17%), dendritic shafts (43.8–58.5%) and dendritic spines (20.8–46.3%) for all three cells. Only one postsynaptic target was an axon initial segment. Small dendrites that do not contain mitochondria in the plane of the section are difficult to distinguish from spines not containing a spine apparatus; therefore some postsynaptic elements had to be followed through 5–20 sections. Even so, some postsynaptic elements could not be confidently placed in either category (Table 1).

Postsynaptic somata. These were remarkably similar to identified spiny stellate cells. They had a soma diameter of 8–10 μm, round nuclei, thin rim of cytoplasm that was poor in cell organelles and received few, and exclusively type II, synaptic contacts (Fig. 6F, G). The identified clutch cell axon made one or rarely two synaptic contacts with one postsynaptic soma. Thus the 21 boutons found to contact somata were distributed on 19 cells. The same postsynaptic somata also received synapses from unlabelled boutons similar to the identified clutch cell terminals.

Postsynaptic dendrites. These were randomly oriented, small, 0.3–1 μm in diameter and received few boutons other than the identified terminals of clutch cells (Fig. 6A, B). Rarely, spines could be seen originating from the dendrites. Two clutch cells (N_8, N_{10}) each established an autapse with their own dendrite (Fig. 6E).

Postsynaptic dendritic spines. These were identified in serial sections on the basis of their shape, the presence of a spine apparatus and by the absence of mitochondria. All spines receiving a type II synapse from the identified clutch cell bouton also received a type I synapse from another bouton of unknown origin (Fig. 6C, D). Some spines could be traced back to their parent dendrites which were very similar to the dendritic shafts that received synapses from clutch cells.

Identified postsynaptic targets

The fine structural characteristics of the majority of the postsynaptic elements suggested that spiny stellate cells were the major targets of clutch cells. We thoroughly searched the axonal fields of clutch cells to see if postsynaptic cells could be directly identified by Golgi impregnation. Contacts between two Golgi impregnated elements are necessarily rare, but in the axonal arborization of clutch cell N_{10} two neurons were found that were directly apposed to boutons of N_{10} (Fig. 3). One of them was a spiny stellate cell (Pn_1 in Figs 3 and 7A, D) with an ascending main axon that could be traced through layer IVB (Figs 3 and 7A). This cell received three synapses on its soma (Fig. 7B, C) and one synapse on one of its dendritic shafts (Fig. 7D) from clutch cell N_{10} (Table 1). The other identified postsynaptic neuron (Pn_2 in Fig. 3) was a smooth dendritic cell at the border between

Table 1. Postsynaptic elements of layer IVC clutch cells in monkey striate cortex

No. of clutch cell	Postsynaptic structure: Soma	Dend. shaft	Spine	AIS	Unident. element	Total
Random sample						
6	4	12	6	—	4	
7	9	31	11	1	1	
8	8	35	37	—	—	
Total	21 (13.2%)	78 (49.1%)	54 (34.0%)	1 (0.6%)	5 (3.1%)	159 (100%)
Non-random sample						
4	—	3	3	—	—	
9	2	—	1	—	—	
10	6 (4)*	2 (1)*	—	—	—	
Total	8	5	4	—	—	17

*Identified Golgi-impregnated postsynaptic elements.
AIS, axon initial segment.

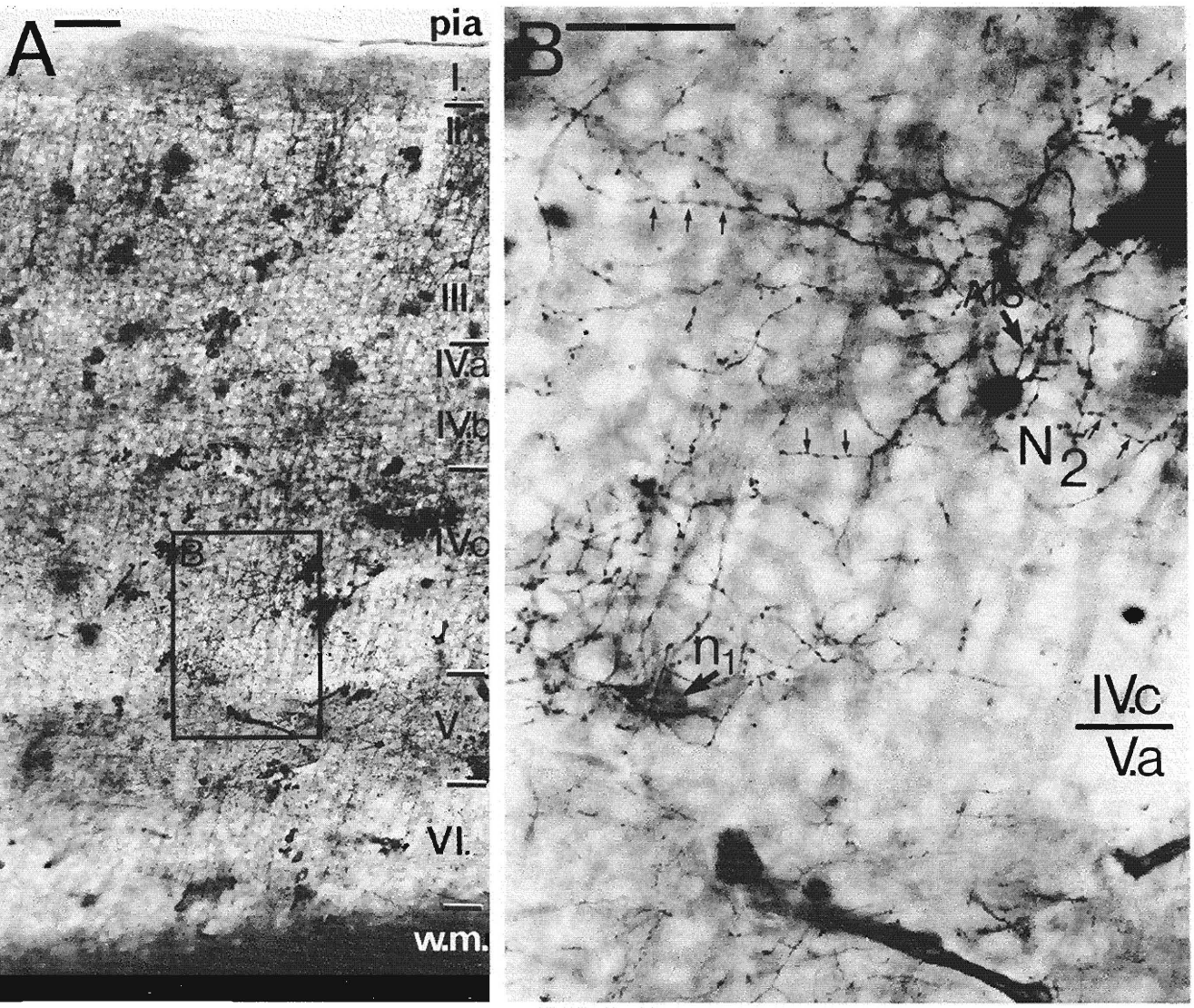


Fig. 1. (A) Light micrograph of a Golgi-impregnated section of monkey striate cortex to demonstrate the layering that has been used in this study. In the framed area there are two clutch cells in layer IVC (n_1 and N_2), which are shown at higher magnification in (B). (B) The lower cell (n_1) was not studied in detail. The upper cell N_2 was GABA-positive and is also shown in Fig. 2. The axon initial segment (A I S) and the characteristic beaded axon collaterals (small arrows) are marked. Bars: (A) 100 μm; (B) 50 μm.

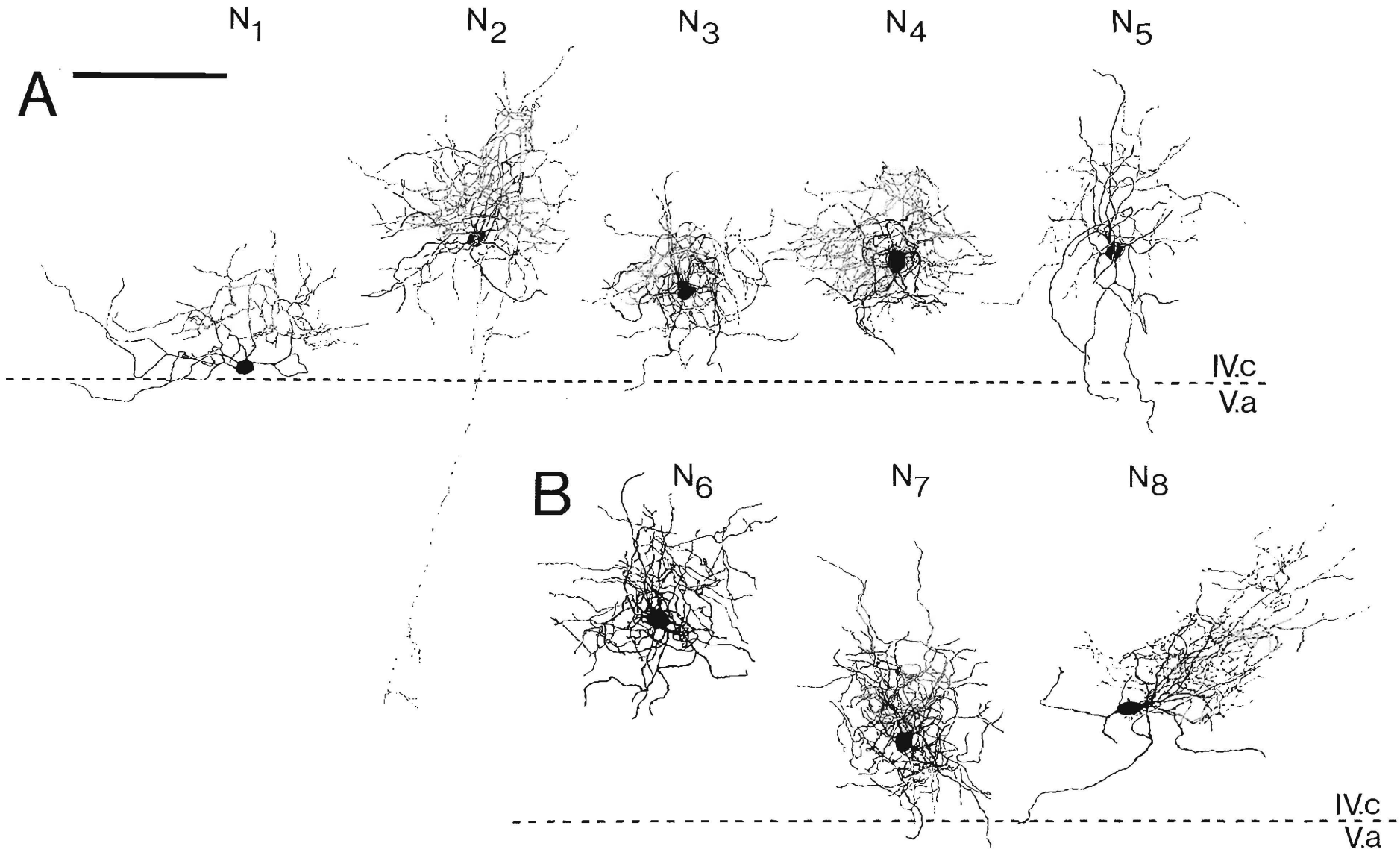


Fig. 2. Axonal (red) and dendritic (black) arborizations of clutch cells (N_{1-8}) in layer IVC of monkey striate cortex. The axon collaterals and the dendritic fields remained largely within layer IVC. All neurons but N_7 (not tested) were shown to be immunoreactive for GABA. Neurons (N_{6-8}) in (B) were selected for quantitative electron microscopic analysis of their postsynaptic targets (see also Table 1). Bar = 100 μm.

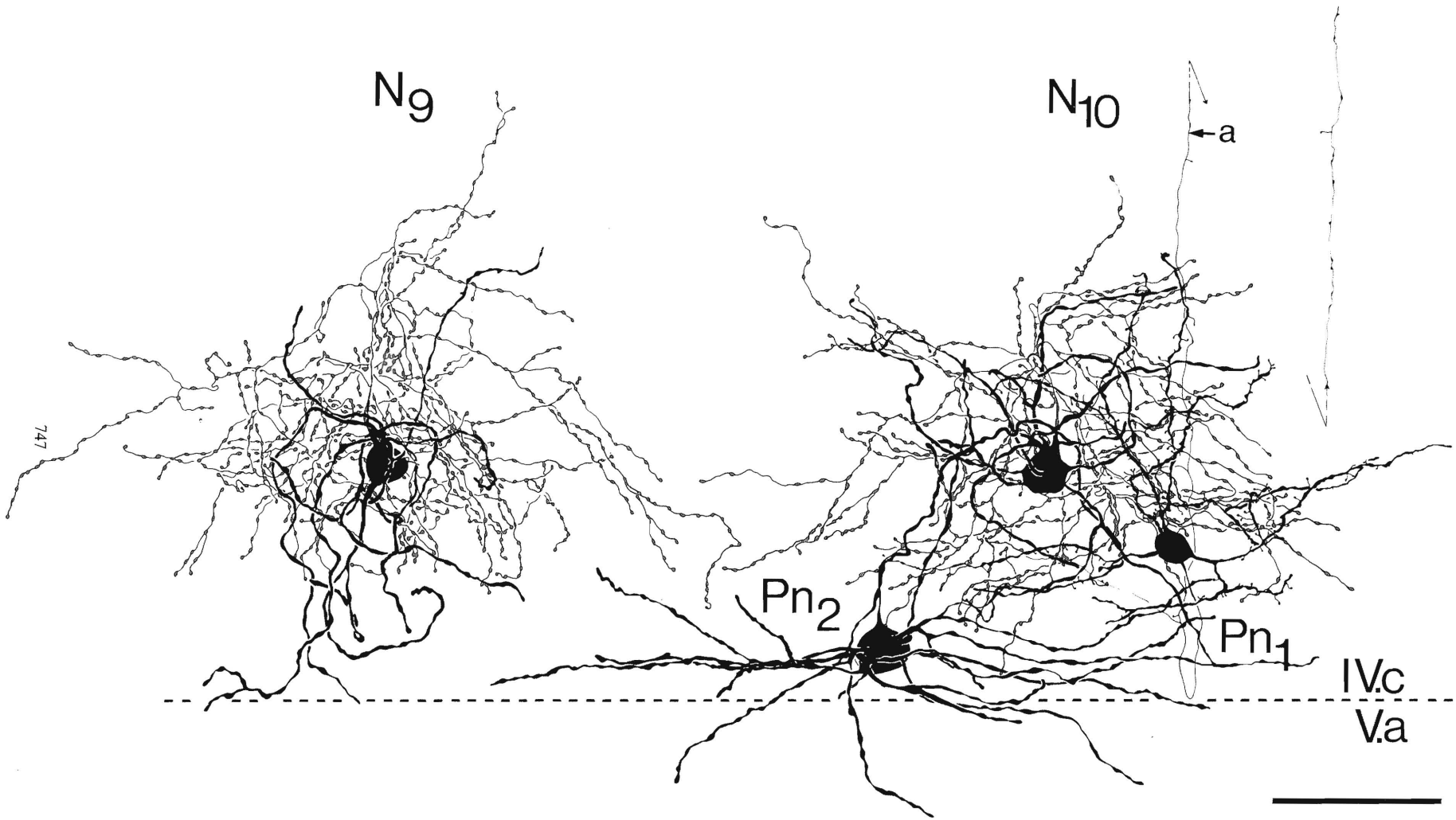


Fig. 3. Drawing of two GABA-immunoreactive clutch cells (N_9 and N_{10}) and two neurons (Pn_1 and Pn_2) postsynaptic to N_{10}. The smooth dendritic cell (Pn_2) was also immunoreactive for GABA (see also Fig. 7E–G). The axon collaterals of (N_9) and (N_{10}) are shown by red. The axon of the postsynaptic spiny stellate cell (Pn_1) gave collaterals in IVC, but the main axon (a) ascended towards layer III. Bar = 50 μm.

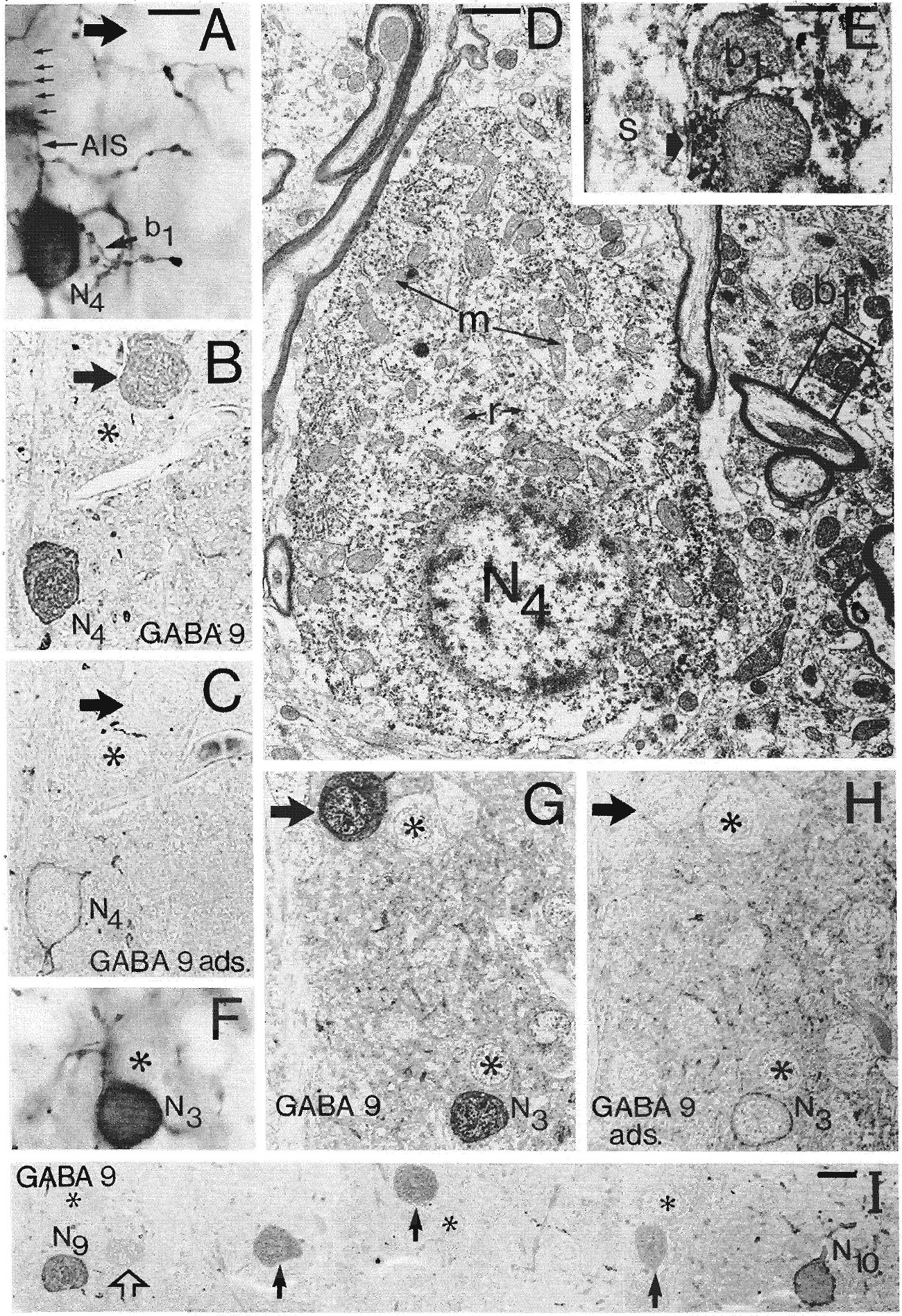
Fig. 4. GABA-immunoreactivity in clutch cells. (A) and (F) Light micrographs of clutch cells (N_4) and (N_3) (drawings shown in Fig. 2). (B) and (C) are 0.5 μm thick consecutive sections of N_4; (G) and (H) are 0.5 μm thick sections of N_3. In (B) and (G) the sections were processed for postembedding GABA-immunostaining and both neurons were GABA-positive. In (C) and (H) the cells were reacted with the same GABA antiserum, but the serum was preincubated with GABA coupled to a solid phase carrier. No immunostaining was seen in these sections. Other GABA-immunopositive perikarya (arrows) and immunonegative somata (asterisks) are also labelled. The ascending axon initial segment (A I S) of N_4 seen in (A) is continued in a myelinated main axon (small arrows), also shown by subsequent electron microscopy. (D) Electron micrograph showing the somata of neuron (N_4) containing a conspicuous amount of ribosomes (r) and mitochondria (m). (E) A bouton (b_1) of neuron N_4, also labelled in (A) and (D) is seen at higher magnification to establish type II synaptic contact (arrow) with a spine (s). (I) Postembedding GABA-immunostaining of a semithin section to reveal the GABA immunoreactivity of neurons (N_9) and (N_{10}). Other immunoreactive perikarya (arrows) having similar size to (N_9) and (N_{10}) and examples of non-reactive neuronal somata (asterisks) are marked. One somata (open arrow) showed only weak immunoreactivity. Bars: (A–C), (F–H) and (I) 10 μm; (D) 1 μm; (E) 0.2 μm.

Fig. 5. (A) and (C) Electron micrographs of clutch cells (N_8) and (N_6) shown in drawing in Fig. 2. The perikarya contain large numbers of mitochondria (m) and receive few synapses (arrows). The nuclei have smooth contours. (B) and (D) GABA-immunoreactivity is shown in semithin sections of both cells as well as in other unidentified neurons (arrows). Some immunonegative cells are marked by asterisks. (E) Electron micrograph of a large bouton forming a synapse (arrows) on the somata of clutch cell N_7. (F) distal dendrite (d) of neuron N_8 receiving two type I synapses (arrows). Bars: (A) 2 μm; (C) 1 μm; (B) and (D) 10 μm; (E) and (F) 0.5 μm.

Fig. 6. Efferent synaptic contacts of clutch cells. (A) and (B) The same clutch cell bouton (asterisks) in serial sections as it makes synaptic contacts (arrows) with two dendrites (d_1, d_2). (C) and (D) Clutch cell boutons establish synaptic contacts (arrows) with spines (s), the most frequent postsynaptic targets. The same spines also receive synapses from boutons of unknown origin (open arrows). (E) A Golgi-impregnated bouton (asterisk) establishes a synaptic contact (arrow) with the dendrite (d) of its parent clutch cell (N_8). (F) and (G). Axo-somatic synaptic contact (arrow) of a clutch cell bouton (asterisk) is seen at low magnification in (F) and the framed area is shown in (G). Note the differences between the postsynaptic perikaryon shown here in (F) and the somata of clutch cells in Figs 4D, 5A and C. Bars: (A–D) and (G) 0.5 μm; (E) 0.2 μm; (F) 1 μm.

Fig. 7. Identified postsynaptic targets of clutch cells. (A) Drawing of a spiny stellate cell (Pn_1) with axon ascending through layer IVB, that received four synaptic contacts from clutch cell N_{10} (see Fig. 3). (B–D) One axo-somatic (b_1 in B and C) and an axo-dendritic (b_2 onto d in D) contact on Pn_1 is shown. The synapses are indicated by arrows. (E) Light micrograph of a Golgi-impregnated smooth dendritic stellate cell, Pn_2 showing GABA-immunoreactivity in (F) and receiving a synaptic bouton (b_3) from clutch cell N_{10} (see also Fig. 3). The same bouton is seen in (G) where it makes type II synaptic contact (arrow) with the soma. A GABA-immunonegative perikaryon (asterisk) is also labelled for alignment. Bars: (A) 50 μm; (B) 2 μm; (C), (D) and (G) 0.2 μm; (E) and (F) 10 μm.

Fig. 8. LGN input and other synaptic contacts of spiny stellate cells. Cells (C_1–C_3) received degenerating geniculo-cortical boutons (db) onto their dendritic spines (s) as shown in (C) and (D), and onto dendritic shafts as in (E). The electron opaque material in the processes of spiny stellate cells is the Golgi deposit. In the case of cell C_3 the dendritic spines found to receive LGN input are numbered 1–5 in (A). The majority of the postsynaptic targets contacted by the axons (a) of the spiny stellate cells were dendritic spines of other neurons. An example is shown in (B) where a spine (s) receives a type I synaptic contact (open arrow) from a bouton (asterisk) of cell C_3, also labelled "B" in (A). Frequently, the degenerating geniculate terminals (db) established synapses (arrows in D) with more than one postsynaptic element. (F) The soma of the Golgi-impregnated spiny stellate cell C_2 is shown receiving synapses (arrows) from boutons having very similar characteristics to identified clutch cell terminals. Bars: (A) 50 μm; (B–F) 0.2 μm.


A
AIS
b1
N4
B
N4
GABA 9
C
N4
GABA 9 ads.
D
m
r
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E
b1
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Fig. 4.

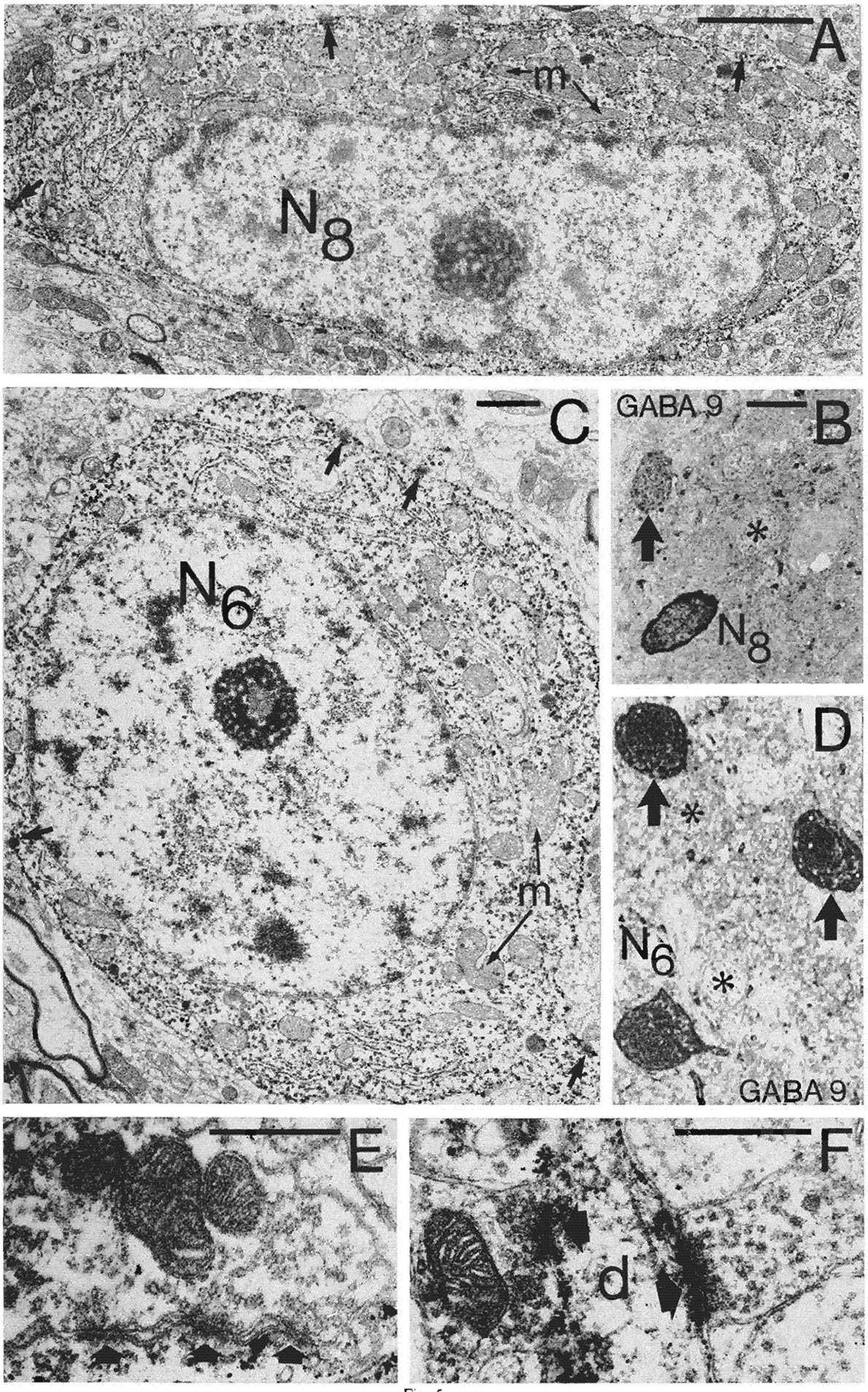

Fig. 5.

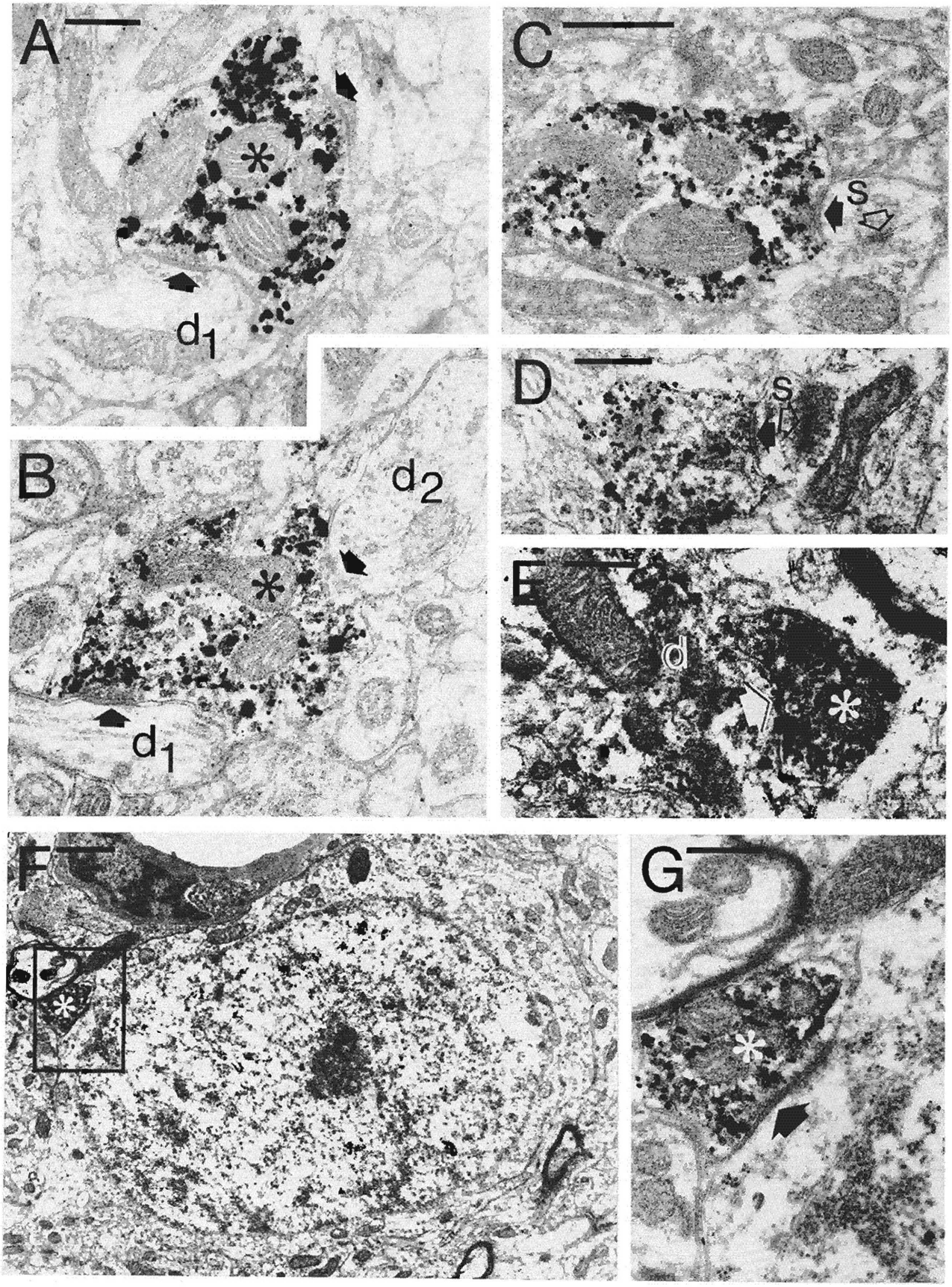

Fig. 6.

layers IV and V. This cell received only one synapse from the clutch cell on its soma (Fig. 7E, G). It had a horizontally oriented dendritic field, and an axon initial segment pointing towards the pia and continuing as a myelinated axon. When tested by post-embedding immunocytochemistry, this cell was immunoreactive for GABA (Fig. 7E, F). Since the clutch cell (N_{10}), providing the presynaptic bouton, was also GABA-positive (Fig. 4I), both pre- and postsynaptic neurons may use the same putative inhibitory transmitter.

Possible inputs of clutch cells

Recent results obtained in the cat demonstrate that LGN afferents monosynaptically terminate on GABA-immunoreactive neurons in layer IV, some of which may correspond to clutch cells described above.[12] Therefore we serially sectioned the dendritic arborization of a clutch cell (N_{11}, Fig. 9) from an area where layer IV contained a very high density of degenerating boutons resulting from an electrolytic lesion of the ipsilateral LGN that destroyed the caudal part of the nucleus projecting to dorsolateral striate cortex from which the cell was taken. About two thirds of the dendritic arborization and the whole perikaryon were serially sectioned and studied, but no degenerating terminal contacted the clutch cell, despite the presence of numerous degenerating terminals in the immediate vicinity. The failure to demonstrate contacts between degenerating terminals and this clutch cell is unlikely to be a result of the Golgi–electron microscopy method employed, because contacts were readily demonstrated on spiny stellate cells (see below).

Another possible source of input to clutch cells could be the rich local axon arborization of spiny stellate cells in layer IVC itself. Preliminary studies were carried out to identify the postsynaptic targets of boutons from three spiny stellate cells in layer IVC. Fourteen boutons were serially sectioned; thirteen of them established contacts on spines (Fig. 8B) and one of them terminated on a dendritic shaft. Thus, spines seem to be numerically the most abundant target as also described recently by Saint-Marie and Peters.[54]

Lateral geniculate nucleus input to spiny stellate cells

The above results demonstrate that the main targets of clutch cells in layer IV are spiny stellate cells, and the presence of GABA in clutch cells suggests that they provide an inhibitory input to spiny stellate cells. The presence of their synapses not only on the soma but also on the dendritic shafts and spines of spiny stellate cells indicates that the input from clutch cells will influence other inputs to the same neuron. The most obvious such input is from the LGN, therefore we studied the distribution of degenerating LGN terminals on three impregnated spiny stellate cells (Fig. 8) that were in the area of dense degeneration, similar to that demonstrated in previous studies.[15,74] The vast majority of the synaptic boutons contacting the dendrites and all the boutons on the soma showed normal structural characteristics. Some terminals were electron-dense and shrunken as described by others studying the effect of LGN lesions,[74] indicating that the boutons were undergoing degeneration. Two dendrites of cell No. 1 (C_1 in Fig. 8) were studied and one degenerating terminal was found on a spine. About half of the dendrites that were within the 90 μm thick section of cell No. 2 (C_2 in Fig. 8) were serially sectioned but only two degenerating terminals were found in synaptic contact with two spines. The third cell (C_3 in Fig. 8) was studied in most detail. About three quarters of its dendrites that were within the 90 μm thick section were recovered for electron microscopic analysis. Five degenerating LGN terminals were found on this cell (see drawing in Fig. 8), four on spines and one on a dendritic shaft (Fig. 8C–E). Although no attempt was made to count all terminals making synapses on this cell, the degenerating terminals constituted only a few percent of all synaptic boutons. The soma, dendritic shafts and more infrequently the spines of this cell received synaptic boutons that in every respect were identical to identified clutch cell boutons.

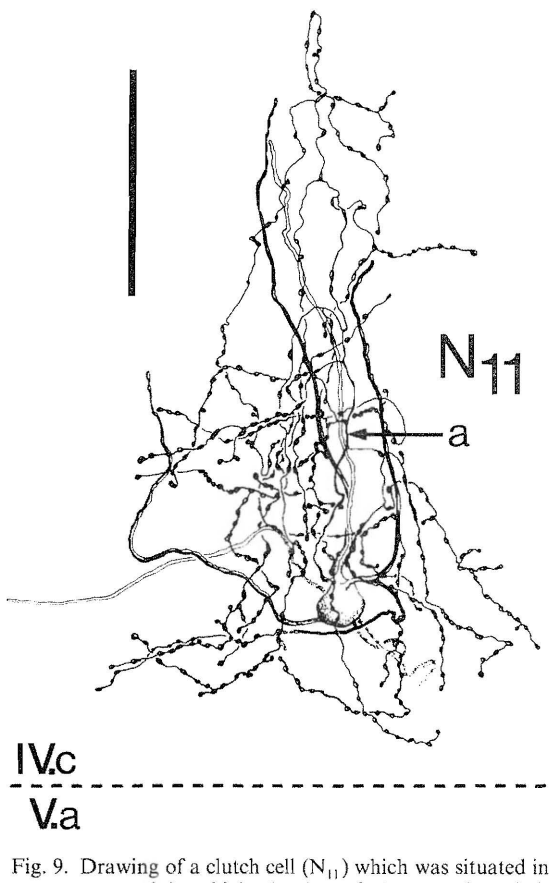

Fig. 9. Drawing of a clutch cell (N_{11}) which was situated in an area containing high density of degenerating thalamocortical axon terminals. No synaptic contacts could be demonstrated between the degenerating boutons and the soma or the dendrites of this cell although most of its processes were examined by electron microscopy. a = axon, Bar = 50μm.

It is worth noting that most of the degenerating LGN terminals in synaptic contact with an identified Golgi-impregnated profile of a spiny stellate cell also made additional synaptic contacts with non-impregnated postsynaptic elements (Fig. 8D). Most of them were dendritic spines, and one LGN bouton could contact up to six spines. Of the 8 boutons that contacted identified elements only one postsynaptic element was impregnated in every instance, indicating that the others belonged to other neurons.

DISCUSSION

Defining features of clutch cells

The name "clutch cell" was suggested[31] for a type of smooth dendritic neuron in the visual cortex of cat found first by Golgi impregnation[41] and described recently in detail using intracellular horseradish peroxidase (HRP) filling.[31] In the cat their dendrites and 90–95% of their synaptic boutons reside in layer IV, and provide type II synaptic contacts on dendritic shafts (35–50%), dendritic spines (30%) and somata (20–30%). Most of their targets are probably spiny stellate, star pyramidal and pyramidal cells of layer IV in an axonal field that closely matches a single discreet patch in the terminal field of the geniculocortical axons.[16,31]

The results of the present study, together with previous descriptions, can now be used to delineate a population of similar smooth dendritic cells in the striate cortex of the monkey. We confirmed previous descriptions[37,40,44,72] that the dendrites and most of the axonal field are restricted to layer IVC, making this neuron one of the more circumscribed cells in the cortex. A principal new finding is that these smooth dendritic cells in layer IV contain GABA. They also form type II or symmetric synaptic contacts as shown previously[44] and confirmed here. The quantitative distribution of the postsynaptic targets shows that dendritic shafts and spines are their major postsynaptic elements and neuronal somata are contacted only infrequently. These general properties are so similar to those of the clutch cells described in the cat that we use the same name in the monkey.

Differences between clutch cells in cat and monkey

The first conspicuous difference is the lateral spread of the axon in layer IV, which is about $300 \times 500\ \mu m$ in the cat[31] but only $100 \times 150\ \mu m$ in the monkey. This could be a result of the different methods used for visualization of clutch cells, because HRP-filled neurons can be reconstructed completely from several sections whereas the processes of Golgi-impregnated cells are usually lost at the surface of a single section. Therefore in the monkey we carefully studied the distance from the soma of all the endings of axon collaterals that terminated naturally within the section using several planes for sectioning. As all the collaterals with natural ends terminated within about 100–150 μm of the soma we conclude that the lateral spread of the axons in the cat and monkey genuinely differ. As will be discussed below the difference between the two species in axonal size may well be related to similar size differences in the terminal fields of afferents from the LGN.

It is noteworthy that although these cells have been described by several authors in the monkey,[44,70,72] and are very frequently encountered in our material, in the cat the only Golgi description is that of Lund *et al.*,[41] who studied young kittens. This discrepancy may well result from the invariable myelination of the axon in the adult cat,[31] as opposed to the monkey, where myelinated main axons have rarely been seen. One further difference that is very likely accounted for by the limitations of the Golgi method is the apparent paucity of interlaminar projections in the monkey. In the cat all HRP-filled clutch cells had a small descending projection through layer V to layer VI, and some had a small projection to layer III.[31] These longer collaterals were myelinated so they would not be revealed by the Golgi deposit. Indeed on several, but not all, cells we observed major radial axon branches whose impregnation ceased abruptly within the section, suggesting that they became myelinated. In only one case could we trace a collateral to layer VI, confirming the interlaminar projection demonstrated earlier for some of these cells.[37] Projection from IVC upwards, at least as far as IVA, has also been illustrated in a schematic form (see Fig. 9–5 in Ref. 42). Thus it is possible that future visualization by intracellular filling will reveal subpopulations of these cells differing in the arborization of their axons.

GABA as the possible transmitter of clutch cells

In the cat it was conjectured that clutch cells may be GABAergic on the basis of their structural similarities to GAD- and GABA-immunoreactive neurons.[31] The present study in the monkey provides more direct evidence by demonstrating immunoreactive GABA in these cells. Clutch cells in the monkey are thus the second identified type of neuron in the cortex in which a putative transmitter has been directly demonstrated, following the demonstration of both GAD[11] and GABA[65] in chandelier cells. If clutch cells not only contain but also release GABA then their effect is probably inhibitory since iontophoretically applied GABA inhibits cortical neurons,[20,34,57,59,60] and GABA antagonists lead to marked excitation.[7,9,53,57,60,71]

Postsynaptic targets of clutch cells

The spectrum of postsynaptic elements in the cat and monkey is very similar, the only notable difference being the higher proportion of axosomatic contacts in the cat[31] as opposed to the monkey, i.e. 20–30% and 13%. On the basis of Golgi–electron microscopic study Mates and Lund[44] emphasized the termination of clutch cells on the somata of spiny stellate neurons in the monkey. Although the terminals of clutch cells can contact the somata of other

neurons and this is conspicuous when viewed in the thick Golgi sections, only about 13% of the targets were somata when a random sample was analysed. Quantitative analysis may therefore modify general impressions. We confirmed that the majority of somata had the characteristics of spiny stellate cells as described by Saint-Marie and Peters,[54] and also provided direct evidence by demonstrating contacts onto an identified spiny stellate neuron. As in the cat[31] other GABAergic cells were a rare target of the GABA-immunoreactive clutch cells in the monkey.

It was not expected that this GABAergic interneuron would direct more than 86% of its terminals onto dendritic shafts and spines. Most of these probably belong to spiny stellate cells, as demonstrated directly in one instance. Each spiny stellate cell is likely to receive a converging input from several clutch cells because the impregnated boutons originating from an identified clutch cell comprised only a small fraction of the type II synaptic input on any postsynaptic cell. Thus in spite of the dense axonal field of clutch cells the identified dendrite received only one synaptic contact, and in other cases we or others[72] observed, without obtaining electron microscopic evidence, that the number of contacts was only one or two. What effect could such a sparse peripheral interaction have on the firing of the postsynaptic spiny stellate cell? On theoretical grounds the ideal site for inhibition of the discharge of the neuron is on the soma or axon initial segment where the action potential is generated.[28] Thus it is unlikely that individual clutch cells with their few GABA-releasing terminals on spines and distal dendritic shafts would substantially influence the threshold of the neuron as measured at the soma. However, the termination on spines and individual dendritic shafts provides an ideal mechanism for selective inhibition of other inputs to the same structures.[28,32,33] To consider this further we need to know what other inputs are received by spiny stellate cells.

Convergence of clutch cells and lateral geniculate nucleus afferents onto spiny stellate cells

When the total axonal field of a clutch cell in the cat was rotated by computer and viewed from the direction of the pia a striking similarity was noticed in the shape, banding and dimensions of the clutch cell[31] axon and LGN axon arbors in the same layer.[13,16,27] Together with the strong preference of clutch cell axons for layer IV, this indicated that an association might exist between the two sets of terminals. The present results further support this hypothesis. Firstly, the size, shape and even the bouton density of clutch cell axons closely matches those of parvocellular LGN afferents terminating mainly in lamina IV$C\beta$.[5] The lateral spread of parvocellular LGN axons is about 150–200 μm and only a few collaterals enter lamina IV$C\alpha$. Although the axons of clutch cells more extensively enter the lower half of lamina IV$C\alpha$ it is possible that here the clutch cell axons seek dendrites of spiny stellate cells that do not seem to respect the boundary between sublaminae α and β.[38,44] While the correspondence between parvocellular axons and clutch cell axons is suggestive, magnocellular axons terminating in lamina IV$C\alpha$ have much larger lateral spreads, about 500–900 μm,[5] i.e. several times the dimension of clutch cell axons. Perhaps it is noteworthy that clutch cells residing in the upper part of lamina IVC have not yet been demonstrated. Either they do not exist or, because of the larger spread of the LGN axons, their axons may be more extensive, requiring myelinization with the consequent absence of impregnation by the Golgi method. If they do not exist, this may be related to the strikingly different receptive field properties of cells in these two layers in the monkey and in the two populations of LGN afferents that innervate them.

The second level of correspondence between LGN and clutch cell axons is in their postsynaptic elements. As demonstrated by our random sample the main targets for clutch cells are spines and dendritic shafts, most likely of spiny stellate origin. The major postsynaptic target of LGN terminals in IVC is also spines and to a lesser extent dendritic shafts,[15,74] and we find here that many of the structures postsynaptic to LGN terminals originate from spiny stellate cells. This makes it very likely that the action of the two sets of converging terminals is related; e.g. the excitatory action of LGN boutons is modulated by the GABAergic, presumably inhibitory action of clutch cell synapses.

On a note of caution it must be pointed out that the input to spiny stellate cells from LGN and from clutch cells was demonstrated in separate experiments. Furthermore the anterograde degeneration method has limitations in revealing the extent of the input from the LGN to single cells because at any particular post-lesion survival time not all the affected terminals may be recognized as degenerating. Clearly further experiments are required to demonstrate directly the extent of convergence of the two inputs to the same spiny neuron.

Clutch cells and GABA-mediated receptive field properties

In proposing functions for clutch cells, it is pertinent to examine the receptive field properties of layer IVC neurons in the monkey in relation to properties known to be influenced by GABA. In doing so we assume that the majority of cells in lower layer IVC receive clutch cell input, which, considering the ubiquitous presence of symmetrical synapses on all parts of spiny stellate cells,[44,54] and the density of the boutons supplied by a single axon, is not unrealistic. Although the axon of a single clutch cell may terminate in both laminae IV$C\alpha$ and β it is probably sufficient to examine the physiological properties of β cells since they show a narrower range of variation. Most of the cells in IV$C\beta$ in monkeys have non-oriented receptive fields, making it very unlikely that

clutch cells are involved in setting-up orientation or directional selectivity and length preference, properties that in the cat strongly depend on GABA-mediated inhibition[53,58,59,61,71] (for review see Ref. 57). Ocular preference is also modified by the application of the GABA antagonist bicuculline in the cat.[61] Thus in layer IVC, where all cells are monocular,[25] GABA-mediated lateral inhibition could in principle contribute to the suppression of responses to the non-preferred eye. This has not been tested in the monkey, but even if present it is unlikely that clutch cells are a major participant. Their axon is confined to within 200 μm of the soma, where the dendrites are also situated, but the width of an ocular dominance column in IVCβ is about 500 μm.[19,24,25] Unless clutch cells received intracortical input selectively from the opposite ocular dominance column, which would require an extraordinary specificity, both clutch cells and their postsynaptic cells are probably innervated by the same eye. Thus none of the properties that have so far been shown to involve GABAergic mechanisms in the cortex are likely to involve clutch cells.

Possible functional role(s) of clutch cells

The fact that the cluch cell arborizes within such a small region of cortex makes it particularly well suited to mediate interaction between cells concerned with the same, as opposed to adjacent, parts of the visual field. Thus, clutch cells may be involved in generating or sharpening the prominent colour-opponency of cells in layer IVCβ in monkeys. In particular, Michael[47] suggested that cells similar to our clutch cells could contribute to the building of double-opponent receptive fields in layer IVCβ. He found that single-opponent colour cells intracellularly injected with HRP were always smooth dendritic cells with beaded axons. On the other hand spiny stellate cells, shown by us to receive input from clutch cells, had double-opponent red/green receptive fields. Thus, as proposed by Michael,[47] double-opponency may be created by the inhibitory action of clutch cells. However, while clutch cells are present in the cat, colour-opponency is absent, thus other functions may be more relevant.

Changes in inhibition by clutch cells could also be responsible for variation with dark adaptation in the size of the centre of concentrically organized or S-type receptive fields of cells in layer IV.[8,55] Additionally a decrease in inhibition could contribute to the reduction or abolition, with dark adaptation, of the spatially separate "on" and "off" regions, such that a cell now gives both "on" and "off" responses over the entire receptive field.[8] This phenomenon is slight in the retina[3] (for review see Ref. 56), increased in LGN[29,73] and apparently much more prominent in cortex.[8] The increase in the overlap of subfields could be interpreted as an expansion of one or the other mechanism. Similarly, both in the visual[71] and in the primary somatosensory cortex[9] of cat, receptive fields of certain classes of neurons enlarged when GABA-ergic inhibition was reduced by the application of bicuculline.

Another possibility, suggested by the remarkable association of clutch cells with layers and neurons that receive input from the LGN, is that they gate, through GABA-mediated inhibition, visual input to the striate cortex. This would also explain the descending projection to layer VI present in the cat,[31] also detected in one of our clutch cells in the monkey and strikingly illustrated by Lund in one of her cells (Fig. 27 in Ref. 37). This projection is precisely positioned radially beneath the clutch cell where the pyramidal cells that project up to layer IV above are located. Thus it is possible that clutch cells not only gate the LGN input to layer IV, but also influence the input to layer IV from layer VI that modulates the activity of layer IV cells at the same retinotopic location.

What activates clutch cells?

At least one intracellularly injected clutch cell has been shown by electrical stimulation to receive monosynaptic LGN input in the cat.[43] With the degeneration method we could not demonstrate LGN input in the monkey but considering that only one cell was examined at one survival time, it is still possible that the LGN provides a direct input. It has been suggested that recurrent collaterals of layer VI pyramidal cells terminate on dendrites of putative inhibitory interneurons,[45] but neither the chemical nature nor the identity of the cells has been determined. A third possibility is that clutch cells are activated by the local collaterals of spiny stellate cells that provide asymmetrical synapses in layer IVC itself.[35,44,54] The major targets of these boutons are spines, but clutch cells could also receive spiny stellate input, because dendritic shafts similar to those of clutch cells (present study) are also contacted,[54] although less frequently. It is possible that clutch cells receive input from all three major layer IV excitatory systems discussed above, thereby detecting overall excitatory activity and through inhibition raising the threshold of their target cells accordingly, to maintain sensitivity and the precision of the responses over a wide range of input intensities to layer IV. To determine the input of clutch cells, and indeed the balance of inputs to the cells in the recipient zone of LGN afferents, is an important next step. What is clear is that they are not innervated by the back projections from visual areas V2, V3, and V5 to area 17 because these pathways are not directed to layer IVC.

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