

A new type of specific interneuron in the monkey hippocampus forming synapses exclusively with the axon initial segments of pyramidal cells

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By means of Golgi staining and gold-toning, we have found an interneuron in the pyramidal cell layer of the hippocampus which forms synapses exclusively on the axon initial segments of pyramidal neurons. An individual initial segment receives up to 30 symmetrical synapses from one axo-axonic cell. Each axo-axonic cell is in synaptic contact with the axon initial segments of several hundred pyramidal neurons. This interneuron is thus ideally situated to synchronise the output of a large population of pyramidal cells and so might be involved in the generation of rhythmic activity and in epileptogenesis.

The hippocampus was one of the first structures in the central nervous system where synaptic inhibition could be demonstrated by electrophysiological methods¹³. It has been suggested³ that the neuron responsible for the inhibition is the basket cell, first described in the classical studies of Ramon y Cajal²¹; this cell is thought to terminate on the perikarya of pyramidal neurons. Later pharmacological studies led to the conclusion that the chemical mediator of the inhibition was γ -aminobutyrate (see ref. 4). It should be noted, however, that the structural basis of the electrophysiological inhibition was deduced from several independent lines of research and is largely inferential (see ref. 14). We now report the identification of a new, highly specific type of interneuron which establishes multiple synaptic contacts exclusively with the axon initial segments of many pyramidal cells. Since the axon initial segment is believed to be the main site of initiation of the action potential³¹, the terminals of this type of interneuron are ideally situated to exert direct control over the firing of a large population of pyramidal neurons.

The brains of adult male rhesus monkeys (*Macaca mulatta*) were fixed by perfusion with a

picric acid-aldehyde fixative³⁰ after the animals had been sedated with ketamine and then deeply anaesthetized with pentobarbitone sodium. The animals had been extensively used in behavioural experiments but there was no reason to assume that the hippocampal formation would be abnormal. Slices of the hippocampus were processed for Golgi staining²⁶ and 90 μ m thick sections were gold-toned^{10,28,29} for correlated light and electron microscopic analysis of identified structures.

In the light microscope, striking clusters of a new type of impregnated axonal arborization were observed (see the camera lucida drawing, Fig. 1), which resembled the axons of axo-axonic interneurons first identified in the visual cortex²⁵. In 8 cases the main axon could be traced back and shown to originate from local neurons whose perikarya were located in the upper part of stratum pyramidale. The neurons were 15–25 μ m in diameter and were usually ovoid or spindle shaped. Their dendrites were mainly orientated parallel to the apical dendrites of pyramidal neurons but were much thinner (Fig. 1).

The most conspicuous feature of the axon is that all its collaterals provide characteristic ter-



Fig. 1. Drawing of a single Golgi-stained axo-axonic cell in the CA1 region of the monkey hippocampus. The upper and lower extremes of the very extensive axonal arborization mark the borders of the stratum pyramidale. The axon (a) gives off several main collaterals, each of which provides vertical terminal segments which appear to lie in the position of the initial segments of pyramidal cells. This type of axonal arborization is quite different from that of the basket cell already described in the literature^{1,21}. We have not shown all the 202 terminal rows of varicosities that could be seen in the microscope because some would have been superimposed; furthermore, only a few of the pyramidal cells are shown (asterisks). Two pyramidal cells, labeled 1 and 2, are illustrated in Fig. 2. One possible reason why this type of interneuron has not been described before in the many studies using similar procedures is the unpredictable nature of the Golgi method; however, it is also possible that the new type of fixative used in this study is partly responsible. Scale: 50 μ m.

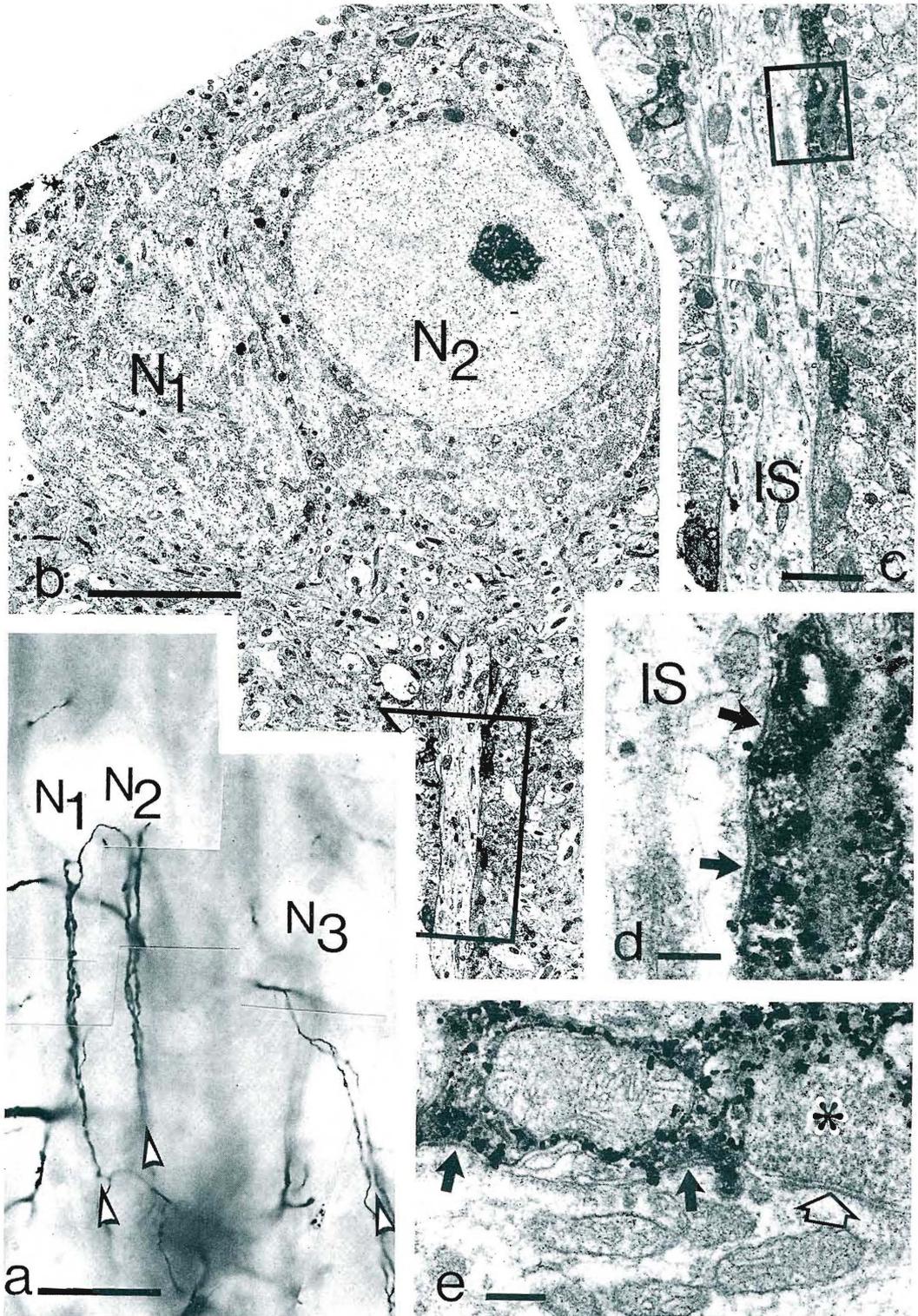


Fig. 2. a: light micrograph showing 3 pyramidal neurons (N_{1-3}) in the CA1 region of the hippocampus, together with the stained vertical terminal segments (arrowheads) of axon collaterals of the axo-axonic cell illustrated in Fig. 1. The terminal axon segments situated under the pyramidal cell bodies appear to surround and follow the axon initial segments. This was established by examination of the same axons and pyramidal cells in the electron microscope (b-e). b: low magnification view of two of the pyramidal neurons (N_1 , N_2). The axon initial segment of N_2 is in the plane of the section (framed area) and is shown in c. c: several Golgi-impregnated, gold-toned boutons (identifiable by the highly electron-dense particles of gold) belonging to the axo-axonic cell are in contact with the initial segment (IS); one of them (framed) is shown at higher magnification in d. d: the Golgi-impregnated bouton makes two symmetrical synapses (arrows) with the initial segment (IS). e: convergence of an unstained bouton (asterisk) and a bouton from the stained axo-axonic cell, both in symmetrical synaptic contact (arrows) with the axon initial segment of the pyramidal neuron N_2 . Scales: a, 25 μm ; b, 5 μm ; c, 1 μm ; d, e, 0.2 μm .

minal segments consisting of one or more rows of varicosities 40–80 μm long. The collaterals form such rows of terminals all along their arcadic course as they pass among pyramidal neurons. Several thin collaterals from different parts of the axon arbor may converge to build up a single terminal segment. The axon arbor remains strictly in the stratum pyramidale and its tangential spread is about 300–500 μm . Because of the difficulty in following a thin axon from section to section only partial reconstructions could be made. The neuron in Fig. 1 was reconstructed from two sections and formed 202 terminal segments, but some of the collaterals were cut so that the total number must have been higher. Each of the terminal axon rows is situated at the base of a pyramidal neuron and is orientated in a way which suggests that it is associated with the emerging axon of the pyramidal neuron (Fig. 2a).

The latter has been established by electron microscopic examination of some of the identified terminal axon segments (Fig. 2b–e). Each impregnated varicosity forms one or two symmetrical synaptic contacts with the axon initial segment (AIS) of a pyramidal neuron. The initial segment was identified by its characteristic origin at the base of the pyramidal neuron and by the microtubule fascicles and membrane undercoating (Fig. 2b,c) unique to this part of the neuron. Since the identified boutons of the interneuron are presynaptic to the pyramidal neuron's axon, we call this neuron an 'axo-axonic cell', as with similar cells identified in the neocortex^{25,27,29}. The terminal segments of two axo-axonic cells were studied. Sections of twelve terminal rows were obtained from the neuron shown in Fig. 1 and 6 from another neuron. From a terminal segment only some of the boutons were studied and from the 2 cells 59 and 69 synaptic contacts were identified, respectively. All of the synapses involved the AIS, indicating an absolute specificity for the postsynaptic target. It was estimated that a single axo-axonic cell provides about 8–30 synapses to a single AIS of an individual pyramidal neuron. The same initial segments received a large number of similar synapses from unstained terminals (Fig. 2e). Because the Golgi procedure usually stains a pro-

cess completely, the unstained terminals probably represent the boutons of other axo-axonic neurons converging onto the AIS. This is supported by the observation that when overlapping axonal arbors of several axo-axonic neurons are impregnated the region around the presumed AIS becomes much denser. Although the degree of convergence has not been established, the detailed electron microscopic study of Kosaka¹⁷ indicates that up to 143 symmetrical synapses may be found along one pyramidal cell AIS in the rat. However, in the latter study the cells of origin of the boutons were not identified. In the monkey, both the stained and unstained terminals contain small clear pleomorphic vesicles and large granulated vesicles and the synaptic zones are symmetrical (Fig. 2d,e).

The above findings agree well with those observed for axo-axonic cells (originally called 'chandelier cells'³⁴) in the neocortex^{11,25,27,29} and make it likely that axo-axonic cells evolved to fulfill a universal role associated with the structural design of pyramidal neurons, rather than with a particular brain area. A possible clue to this universal function is that the AIS is generally considered as the region responsible for generating the axonic axon potential³¹. Whereas synaptic control of neuronal firing at the level of the soma and dendrites is graded, that at the level of the AIS might well be 'all or nothing'⁶. Thus it is likely that an interneuronal system providing a powerful input specifically to this part of the neuron could influence the firing of the neuron in a very direct way.

We can only speculate about the nature of the control exerted by the axo-axonic cell in the hippocampus. By analogy with the basket cell synapses on Purkinje cell axon initial segments^{7,15} and with the synapses on the AIS of the Mauthner cell⁹, an inhibitory effect would be the most obvious function. While in these two cases the structural design is different and indicates that electrical as well as chemical inhibitory mechanisms may operate, in the hippocampus the nature of the axo-axonic contacts suggests a chemical interaction. A possible inhibitory transmitter is γ -aminobutyrate and it is noteworthy that glutamate decarboxylase is present in the pyramidal

cell layer³³, although it has so far only been reported to occur in axo-somatic boutons²³; further work is required to see if it is present in the boutons forming synapses on the AIS of hippocampal pyramidal neurons as it is in the neocortex¹⁹.

The most important feature of the potential interaction between axo-axonic cells and pyramidal cells is that each axo-axonic cell forms synapses with several hundred pyramidal cells at a site where its input will not have to be integrated with other inputs on the soma and dendrites. Thus, the output of a single axo-axonic cell could simultaneously influence a large number of pyramidal cells in the same way: pyramidal cells would be simultaneously either inhibited or released from inhibition. This has two implications for events in the hippocampus that involve the synchronous firing of many pyramidal neurons. First, if the new type of interneuron is the last link in a recurrent inhibitory loop^{2,32}, it could be involved in the generation of rhythmic

activity⁸. Second, as the hippocampus is one of the areas of the brain most prone to seizures¹² and since synchronous firing occurs during seizures²⁰, it is possible that epilepsy might be the result of interference with the normal pattern of control exerted by these interneurons on pyramidal cells; the consequence would be a paroxysmal synchronous discharge in a large population of pyramidal cells. Our findings add a further possible mechanism of epileptogenesis to others that have been proposed, e.g. loss of recurrent inhibitory control by basket cells^{5,24}, extracellular accumulation of potassium ions²⁰, dendritic action potentials^{35,36} and electrotonic coupling between pyramidal cells^{16,18,22}.

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