MORPHOLOGICAL PLASTICITY OF POSTSYNAPTIC NEURONES IN REACTIVE SYNAPTGENESIS

BY JOZSEF HAMORI
Laboratory of Neurobiology, Semmelweis University, Tuzolto u. 58, 1094 Budapest, Hungary

Summary

Partial deafferentation of certain brain regions (septal nuclei, hippocampus, etc.) in adult animals results (1) in the disappearance of degenerating axon terminals and (2) in the short-term persistence of vacant postsynaptic sites. These postsynaptic sites have been shown to be re-supplied by sprouted axon terminals of intact axons. This paper will demonstrate that, in brain regions (e.g. cerebellar cortex, lateral geniculate nucleus) where axonal sprouting of local elements or of persisting afferent axons is negligible or absent, synaptic reorganization involves the active participation of postsynaptic dendritic and somatic elements of surviving local nerve cells. Synaptic regeneration can be demonstrated by morphological means both in developing and in adult central nervous system. The dendrites may show two types of response to deafferentation: (1) the formation of presynaptic specializations along their otherwise 'classical' postsynaptic membrane (the axonization of dendrites) resulting in the formation of new, dendrodendritic synapses, and (2) the 'adaptive' (structural) reduction in size ('atrophy') of the denervated nerve cell dendritic arborization, leading to a relative increase in density of the surviving (though non-sprouting) afferent axon terminals. In both cases a partial functional recovery can be demonstrated.

Introduction

It is generally accepted that certain neurones in the central nervous system (CNS) of adult animals maintain their potential to form new synaptic junctions in response to lesions or other stimuli. It has been demonstrated that partial denervation of septal nuclei (Raisman, 1969; Raisman and Field, 1973), hippocampus (Matthews et al. 1976; Frotscher et al. 1981; Steward and Vinsant, 1983; Hoff, 1986; Steward et al. 1988), red nucleus (Tsukahara, 1981; Murakami et al. 1982) and other CNS regions (Murray et al. 1979; Zimmer et al. 1982; Bromberg et al. 1987; Ichikawa, 1987a,b) was followed by reoccupation of the partially denervated nerve cell by newly formed (collateral) axonal branches of persisting afferent or local fibres, resulting in the formation of 'new' synaptic contacts. This process has been defined (Matthews et al. 1976) as 'reactive' synaptogenesis, implying that it is

Key words: synaptogenesis, dendrites, cerebellum, thalamus.
a reaction to stimuli such as lesions but is not part of the normal developmental process. The emphasis in this type of reactive synaptogenesis is on the active role of axonal processes in finding and ‘resaturating’ the available vacant postsynaptic sites. Previously, ultrastructural studies of reinnervation in the CNS as well as in autonomic ganglia (Raisman and Field, 1973) have suggested that the target neurones have a more or less fixed number of postsynaptic sites, and that no new postsynaptic sites are formed during reactive synaptogenesis. More recently, however, indirect evidence (Matthews et al. 1976; Hillman and Chen, 1981; Somogyi et al. 1982) and direct observations (Purves and Lichtman, 1987) have suggested that mature mammalian neurones maintain a potential to produce new postsynaptic receptor surfaces for new synaptic contacts. They are not, therefore, merely passive participants in reactive synaptogenesis, but are actively involved. The aim of this paper is to present further evidence for the dynamic role of postsynaptic elements during synaptogenesis in certain regions of the adult nervous system. In brain regions such as the cerebellar cortex and the thalamic nuclei, where the neuronal basis of axonal-type reactive synaptogenesis involving the sprouting of local or extrinsic axons is negligible or lacking, synaptic reorganization following partial (Hamori and Silakov, 1980; Somogyi et al. 1984, 1987) or total (Hamori and Somogyi, 1982) deafferentation occurs via the active participation of postsynaptic dendritic processes or even of postsynaptic perikarya. Two types of dendritic reactive synaptogenesis will be described. In the first, the formation of presynaptic specializations along their otherwise ‘classical’ postsynaptic membrane (Hamori and Silakov, 1980; Hamori and Somogyi, 1982; Somogyi et al. 1984; Kalil and Behan, 1987) results in the genesis of new, dendro-dendritic or even somato-dendritic synapses. In the second, the ‘adaptive’ structural reduction in size of the denervated nerve cell dendritic arborization leads to a relative increase in density of the surviving (though nonsprouting) afferent axon terminals (Somogyi et al. 1987).

Deafferentation-induced development of presynaptic dendrites in cerebellar cortex

In normal, intact cerebellar cortices, all nerve cells are classical neurones (Eccles et al. 1967; Palay and Chan-Palay, 1974); the somata and dendrites are invariably postsynaptic, and the axons are exclusively presynaptic. Under abnormal conditions, as in organotypic cerebellar cultures (Kim, 1974) or in cerebellar mutant mice (Sotelo, 1975), however, granule cells were shown to accumulate synaptic vesicles in their somata or in their dendrites. Similarly, following neonatal X-irradiation, Golgi neurones were shown to develop presynaptic sites in their somata and dendrites (Sotelo, 1977).

In another experimental study (Hamori and Somogyi, 1982) it was shown that mossy fibre deafferentation of the granular layer alone results in the synaptic reorganization of cerebellar glomeruli, with the formation of presynaptic sites in
Dendritic type of induced synaptogenesis

The dendrites and somata of granule cells and of numerous (although not all) Golgi nerve cells.

The main synaptic afferents to the granular layer are the mossy fibres. The majority of these are excitatory and utilise glutamate as a transmitter, although some nucleocortical mossy endings have been shown to be GABAergic (Hamori and Takacs, 1989). The mossy terminal in the cerebellar glomerulus is contacted synaptically by 200–300 small postsynaptic dendritic digits of the granule cells (Jakab and Hamori, 1988). In addition, in many glomeruli large ‘hairy’ dendritic processes of the Golgi neurones also receive synaptic contacts from the centrally located mossy terminal (Eccles et al. 1967). Both types of dendrite receive synaptic contacts at the periphery of the glomerulus from small axonal profiles of the Golgi cells (Eccles et al. 1967).

Fifteen to thirty days after surgical deafferentation of the cerebellar cortex the small Golgi nerve cells (but not the large ones) developed presynaptic sites both on their somata and on their thick intraglomerular dendritic processes (Hamori and Somogyi, 1982). Interestingly, the Golgi neurones that developed presynaptic dendrites were, without exception, glycine-containing, whereas those that did not exhibit presynaptic dendrites, usually large Golgi cells, stained only for GABA (R. L. Jakab and J. Hamori, unpublished observations). In any case, the newly developed presynaptic sites faced the dendritic digits of the granule cells; the dendro-dendritic synapses were, occasionally, reciprocal.

Granule cells also frequently developed presynaptic sites, both in their dendrites and in their somata, after mossy fibre deafferentation. Quantitative analysis of synaptic numbers in mossy-deafferented glomeruli has shown that the resulting presynaptic dendritic synapses by granule cells are numerous within the glomeruli. Nearly 50 such synaptic contacts can be found in each deafferented glomerulus. These are mainly dendro-dendritic and are probably excitatory (Table 1; R. L. Jakab and J. Hamori, in preparation).

Of special interest was the finding that, following mossy fibre degeneration, the total number of synaptic junctions in the reorganised synaptic glomeruli did not change significantly (Table 1). The only important alteration was in the proportion

Table 1. Number of synaptic junctions in control and mossy-fibre-deafferented cerebellar glomeruli

<table>
<thead>
<tr>
<th>Number of synapses</th>
<th>Mossy</th>
<th>Golgi axon</th>
<th>Dendro-dendritic</th>
<th>Total number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>145±23</td>
<td>87±33</td>
<td>0</td>
<td>232</td>
</tr>
<tr>
<td>Deafferented</td>
<td>0</td>
<td>201±59</td>
<td>47±9</td>
<td>248</td>
</tr>
</tbody>
</table>

Average of five simple glomeruli in each group.

Dendro-dendritic synapses represent granule-to-granule dendritic contact only, since dendrites of Golgi cells were absent in the simple glomeruli investigated.
of excitatory and inhibitory synaptic junctions. In the normal glomeruli, excitatory synaptic junctions (represented exclusively by mossy terminals) made up the majority of the 232 individual junctions (Table 1); in the reorganised glomeruli, the inhibitory junctions of Golgi axons became the dominant synaptic type, while dendro-dendritic (excitatory) junctions between granule cells represented only one-fifth of all synaptic junctions. The number of postsynaptic dendrites within the mossy-fibre-deprived glomerulus did not change during the first month after deafferentation (Table 2), suggesting that the number of synapses that belonged to the postsynaptic nerve cells remained unchanged, irrespective of the excitatory or inhibitory nature of the new presynaptic elements. A similar observation has been made by Hillman and Chen (1981) in experiments leading to a reduction of the presynaptic parallel fibre/Purkinje cell ratio in the cerebellar cortex. These authors have suggested that there is a constancy in total synaptic area determined intrinsically by the recipient nerve cell. This might also be the case in sympathetic ganglia, where Field and Raisman (1985) have demonstrated that, under normal conditions, postsynaptic ganglion cells have a very limited potential to increase their synaptic surface, even in the presence of an excess of presynaptic partners.

**Deafferentation- and/or axotomy-induced development of presynaptic dendrites in lateral geniculate nucleus of cat**

‘Axonization’, the development of presynaptic sites in partially deafferented relay cell dendrites, has been observed in a specific thalamic nucleus, the lateral geniculate nucleus (LGN) of cats (Hamori and Silakov, 1980; Hamori, 1982; Somogyi *et al.* 1984; Kalil and Behan, 1987). Following chronic disconnection from the cortex (the main source of afferentation to the LGN), it has been found that those geniculo-cortical relay neurones surviving axotomy (about 15 % of the total number of relay cells in the intact LGN: Madarasz *et al.* 1983) developed presynaptic sites on the surface of their dendritic processes (Fig. 1). The new presynaptic dendrites formed synaptic contacts with other relay neurones or with local, GABAergic interneurones. Recent electrophysiological data suggest that, following damage to the visual cortex in adult cats (Tumosa *et al.* 1989), surviving geniculo-cortical relay neurones exhibit abnormally large receptive-field centres. It is suggested that the newly formed dendro-dendritic excitatory synapses between surviving cells, mostly Y-cells, are the morphological basis for the electrophysiological observation.
Dendritic type of induced synaptogenesis

Fig. 1. Induction of presynaptic sites in geniculo-cortical relay cells following chronic cortical deafferentation. Perikarya and dendrites of relay neurones in the control LGN possess exclusively postsynaptic sites (A). In addition to postsynaptic sites, cortically deafferented neurones also develop presynaptic sites (B) (indicated by synaptic vesicles), mostly in their dendrites. Arrows indicate direction of impulse transmission.

Adaptive atrophy of dendrites following visual deafferentation of lateral geniculate nucleus

Chronic visual deprivation of the developing subcortical 'relay' station, the LGN, has been shown to result in a shrinkage of the transneuronally affected geniculo-cortical neuronal perikarya in rats, cats and monkeys. Neurones in the visually deprived LGN lamina become 20–60% smaller following monocular eyelid closure (Garey and Blakemore, 1977; LeVay and Ferster, 1977; Hitchcock and Hickey, 1982; Tigges et al. 1984) or after neonatal enucleation (Robertson et al. 1989). Other studies have demonstrated a shrinkage of the deafferented LGN lamina in the adult monkey (Matthews et al. 1976; LeVay, 1971) as well as in adult cats (Cook et al. 1951; Guillery, 1973; Eysel and Wolfhard, 1984). Apart from the loss of retinogeniculate fibres and terminals, the main morphological event in this transneuronal atrophy in adult animals is probably the shrinkage of neuronal somata. No cell death was found in the visually deafferented lamina up to 163 days after enucleation (Eysel and Wolfhard, 1984). Recently it was found that, not only relay cell perikarya, but also their dendritic arborizations, react to chronic deprivation by specific morphological alterations.

This type of active response of dendrites has been described in the LGN of cats (Somogyi et al. 1987) following chronic visual (retinal) deafferentation. Their study was designed to establish the morphological background of the electrophysiologically observed functional plasticity in the LGN of adult cats after total or
partial retinal lesions (Eysel, 1979, 1982; Eysel and Wolfhard, 1984). In a previous study, Eysel (1979) reported that, after visual deafferentation, the maintained activity of the deafferented relay cells was initially depressed, but recovered significantly after 10 weeks. A total absence of reactive sprouting of retinal axons in adult LGN, which would otherwise provide a possible explanation for the observed functional plasticity following partial retinal lesions, has been established beyond doubt by several authors (Baisden et al. 1980; Eysel, 1982; Guillery, 1972; Stelzner and Keating, 1977). Other possible mechanisms for the recovery of excitability of retinally deafferented geniculo-cortical relay cells should, therefore, be taken into account. These include: (1) the disinhibition due to selective transneuronal degeneration of GABAergic interneurones, (2) axonal sprouting of cortical fibres and (3) dendritic elongation of relay neurones. The second possibility seemed to be particularly attractive since the regained, and even increased, excitatory drive in visually deafferented LGN cells following retinal lesions could also be elicited by electrical stimulation of the visual cortex (Eysel, 1979). Indeed, the cortically elicited response in the deafferented LGN was even enhanced compared with the control. Contrary to expectations, however, investigations disproved all three of these possibilities (Somogyi et al. 1987). It was shown that the regained excitability of relay neurones could not be caused by disinhibition, since quantitative immunocytochemistry revealed that the proportion of GABA-positive and GABA-negative cells, as well as the synaptic density of GABA axons in the LGN, were unchanged even 9 months after retinal deafferentation. The second possibility, that the enhanced excitability of LGN neurones could be the result of sprouting of corticothalamic axons, was also disproved, since quantitative electron microscopic analysis showed that the number of cortical terminals in the retinally deafferented LGN did not change either. Similarly, the third possibility, that elongation of relay cell dendrites led to the re-establishment of functional connections, could not be substantiated; on the contrary, the main and meaningful alteration was a significant numerical decrease of postsynaptic dendrites of both X and Y cells. According to our recent, unpublished data (G. Legrady and J. Hamori, in preparation) this shrinkage of the dendritic arborization is due almost exclusively to a significant shortening of dendritic segments between two branching points, while the number of branches remains at the control level. Because of the shrinkage of the dendritic trees, however, the density of cortical synaptic input to LGN cells becomes elevated by almost 60%. This naturally means that there is a substantial relocation of the cortical terminals along the shrunken dendritic arborization (Fig. 2). It is suggested (Somogyi et al. 1987) that the regained excitability of geniculocortical neurones is at least partly a consequence of this adaptive reduction in size of the dendritic arborization of the retinally denervated neurones. This results in a relative increase in density of the excitatory cortical input per neurone (Fig. 2). It is relevant to mention here that, in the cortical pyramidal cells of ageing rodents, dogs, monkeys and also man (Leuba, 1983; Geinisman, 1979; Machado-Salas et al. 1977; Mervis, 1978; Uemera, 1980; Scheibel et al. 1975), a marked physiological
Fig. 2. Dendritic processes of geniculo-cortical neurones become shorter following retinal deafferentation (denervated synapse) of the LGN, resulting in an increase in the density of cortical terminals to the shrunken nerve cell.

loss of presynaptic neurones is accompanied by a similar atrophy, with a 30–40% decrease in dendritic branches and a 50% decrease in dendritic spines.

Concluding remarks

In addition to classical axonal reactive synaptogenesis, a new type, the dendritic type of reactive synaptogenesis, has been recognized and described. By definition, there is an active participation of postsynaptic dendrites (or even somata) in the induced synaptogenesis that follows deafferentation in the adult nervous system. Two regions where this ‘dormant’ morphogenetic potential of certain neurones has been investigated in detail are the cerebellar cortex and the LGN. In both regions, the reactive development of presynaptic sites in otherwise exclusively postsynaptic dendritic processes (granule cells and small Golgi neurones, in the cerebellar cortex and relay neurones in the LGN) has been described. The new synaptic formations were dendro-dendritic, dendro-somatic, somato-dendritic or even somato-somatic (Hamori and Somogyi, 1982). The other major type of dendritic reaction to deafferentation was observed in the LGN, where there was an adaptive reduction in size (functional atrophy) of relay cell dendrites. In this case, since dendritic atrophy caused by visual deafferentation was accompanied neither by axonal sprouting nor by axonal loss of the main surviving (cortical) afferents, the net result was the synaptic reorganization of cortical endings onto the shortened relay cell dendrites, and, as a consequence, an increased density of this type of excitatory input along the dendrites of relay neurones.

The main factor in eliciting either of these active dendritic structural alterations appears to be the partial (in the LGN) or total (in the cerebellar cortex)
deafferentation of the particular region. The absence of significant axonal sprouting for reactive synaptogenesis in both regions is, at least partly, compensated for either by axonization or by the shrinkage of postsynaptic dendrites. Although a functional study of the synaptically reorganised cerebellar glomeruli is still lacking, electrophysiological investigations of the chronically decorticated LGN (Tumosa et al. 1989) also demonstrate a certain functional recovery within the nucleus, though with more diffuse and abnormally large receptive field centres than in the control LGN. This suggests, that the newly formed dendo-dendritic synaptic links between neighbouring relay neurones are functionally valid. The synaptic reorganization of cortical input to the shrunken dendritic arborizations of retinally deafferented relay neurones in the LGN (Somogyi et al. 1987) provides another example of the active participation of postsynaptic elements in functional recovery (Eysel and Wolfhard, 1984) of nerve cells previously handicapped by partial deafferentation.

References


Dendritic type of induced synaptogenesis


reduce number, size, and acetylcholinesterase histochemical staining of neurons in the dorsal lateral geniculate nucleus of developing rats. Devl Brain Res. 47, 209–225.


