J. exp. Biol. 153, 277–287 (1990)
Printed in Great Britain © The Company of Biologists Limited 1990

SYNAPSE FORMATION IN THE ADULT BRAIN AFTER LESIONS AND AFTER TRANSPLANTATION OF EMBRYONIC TISSUE

BY G. RAISMAN AND P. M. FIELD

The Norman and Sadie Lee Research Centre, Laboratory of Neurobiology, National Institute for Medical Research, MRC, Mill Hill, London, UK

Summary

Some years ago it was demonstrated that when the adult rat septal nuclei are partially deafferented the remaining afferent fibres form new connections. The conclusion that new synaptic connections form in the adult central nervous system (CNS) was greeted initially with much scepticism, later with over-enthusiasm and unwarranted generalisation to all lesion situations, together with even less warranted attribution of various beneficial functional properties.

Today, as the pendulum swings into a more reasonable position, some of the original observations, which at the time attracted little attention, have become more interesting.

(1) The observation that in the normal septal nuclei the ratio of spine to shaft synapses is extraordinarily constant (to an accuracy better than 1%) from one animal to another. How could such almost crystalline rigidity of structure be produced in normal development and maintained in the face of major lesion-induced changes in connectivity?

(2) The observation that synaptic re-occupation by sprouting axons restores exactly the normal number of synapses, presumably indicating that the neurones have a fixed number (as well as spine/shaft distribution) of postsynaptic sites. Thus, the septal lesion paradigm is as strong a method for investigating synaptic rigidity as for investigating plasticity.

In the intervening years, the use of embryo to adult transplantation has made it obvious that considerable reconstruction of adult brain synaptology is possible, and that many of the normal rules of connectivity are maintained (most prominently for the 'point-to-point' axonal systems). What could lead to further fruitful investigation is the extent to which the observations (e.g. relating to hierarchies of axonal preference, the need for denervation, and the involvement of glial cells) in partially deafferented adult systems, such as the septal nuclei, are retained, or modified, in face of the ingrowing fibres from embryonic transplants.

Introduction

Many conceptual advances owe their origin to technical developments, often in an unrelated field. The acceptance of the idea that new synapses can form in the

Key words: synaptogenesis, lesion, septal nuclei, hippocampus, embryonic transplants.

adult brain after injury was largely the result of the application of the then new technique of electron microscopy to the study of neuroanatomy.

It was not that electron microscopy (Peters *et al.* 1976) had confirmed Cajal's brilliant hypothesis of nearly a century ago (which took the form of Waldeyer's 'Neuron Theory') that neurones were separated by a gap, nor was it the new electron microscopic quest for the structural basis of synaptic function (vesicles and postsynaptic densities) that gave the clues to the theory of 'plasticity'. Rather it was a much more humble aspect of electron microscopy – simply that synapses were unitary structures, and could therefore be counted. Combined with the demonstration that axotomy caused electron-dense degeneration of presynaptic terminals, neuroanatomy was moving (to use a computer metaphor) from the analogue to the digital, from a descriptive science to a measuring science. There was now a simple, quantitative measure of the anatomical 'strength' of a neuronal projection.

The usefulness of this approach has been fully vindicated by much subsequent work, and its recent application to the study of synapses formed by transplants suggests its usefulness is by no means exhausted. Indeed, some of its most remarkable findings in the study of normal tissue, came early on, and have still not been fully exploited.

This paper illustrates some of these points by reference to a series of studies from our own laboratory.

Studies of synapse formation after partial denervation in the adult rat septal nuclei

These experiments can be discussed in terms of 10 'principles'.

(1) *Synapses are distributed on different parts of the postsynaptic surface in a highly consistent manner*

One of the first observations to arise from electron microscopy of the hippocampus (Hamlyn, 1963) was that the postsynaptic surface is divided into different regions: the cell body, the dendritic shafts and the dendritic spines. In the septal nuclei, counts of the numbers of synapses on these three regions showed that the vast majority of presynaptic terminals, 63.5 %, made synaptic contacts on dendritic spines, while 32.5 % made contact on dendritic shafts and only 4 % made contacts on cell bodies (Raisman and Field, 1973). What was even more remarkable was the striking consistency of the relative shaft/spine distribution. Less than 1 % variation was observed between quite independent samples from different animals. This uniformity of postsynaptic distribution must be one of the least variable parameters found in any tissue measurement. So far nothing is known of the regulatory developmental mechanisms which could give rise to such extraordinary consistency.

(2) *Presynaptic terminals belonging to axon systems from different sources terminate on different parts of the postsynaptic surface*

The septal nuclei do not present a highly favourable area for studying differences in the distribution of different afferent axons. Far clearer examples are found in the cerebellum (Palay and Chan-Palay, 1974) and hippocampus (Hamlyn, 1963). Nonetheless, we have found one highly significant difference in presynaptic pathway distribution. Using electron microscopy of degeneration to identify the presynaptic terminals of different pathways, we found that fibres of hippocampal origin passing through the fimbria terminated only upon dendritic shafts and spines, never upon cell somata. In contrast, afferent fibres from the medial forebrain bundle to the septal nuclei terminated not only upon dendrites, but also upon cell bodies, where they accounted for about 24 % of all axosomatic terminals (Raisman, 1969*a*). This observation was used, later, to design an experiment to support the idea of deafferentation-induced synaptogenesis (Raisman and Field, 1973).

(3) *The total number of synapses is fixed not only as a result of normal development, but it also remains fixed in the face of partial deafferentation and reinnervation in the adult*

The total number of synapses per area of 100 μm^2 in the normal lateral septal nucleus is close to five. The hippocampal input to the lateral septum consists of axons that arise from all rostro-caudal levels of the hippocampus and are gathered into the fimbria, where they can be totally ablated by a complete transection of the fimbria at the rostral pole of the hippocampus. In the first week after transection of the ipsilateral hippocampal input through the fimbria, the presynaptic terminals belonging to the cut axons undergo electron-dense degeneration. Up to 30 % of synapses degenerate. The number of degenerating terminals is highly consistent from animal to animal, as is their relative distribution on dendritic shafts and spines (Raisman and Field, 1973).

As the time after transection increases, the number of degenerating terminals falls (as a result of the phagocytic action of the adjacent reactive astrocytic processes – see below), until it reaches very low levels by 4–6 weeks after operation. What was unexpected, when we first observed it, was that the number of non-degenerating synapses per unit area increased in exact proportion to the removal of the degenerating synapses. At 4–6 weeks after operation it was close to normal levels and showed no further increase thereafter. The number of neurones per unit area remained constant, indicating that the changes were not due to shrinkage.

The simplest explanation for these observations is that, as the degenerating synapses were removed, their places were taken by new synapses. The fixity of the total number of postsynaptic sites would explain the restoration of normal synapse numbers and, on this hypothesis, is clearly a property of the post- and not the presynaptic elements. Nonetheless, this fixity in numbers of postsynaptic sites

results in a fixity in total numbers of synapses. In other words, plasticity in the presynaptic elements cannot over-ride the constraints determining total numbers of synapses, and the reactions of the presynaptic elements are subservient to constraints acting at the level of the postsynaptic elements.

Where did the new synapses in the septum come from? Histological observation of the cut fimbria confirmed that, as in the case of all cut central axons, the cut fimbrial axons do not regenerate back to the septum. This was easy to see, since the lesion opens the lateral ventricle and results in a clear and permanent separation of the two margins of the cut tract. The question remained, therefore, of how to prove the then seemingly revolutionary idea that new synapses were being formed in adult brain.

(4) *Deafferentation in the vicinity of surviving presynaptic terminals induces synapse formation and leads to abnormal patterns of connectivity*

To prove that new synapses were being formed, we took advantage of the difference in distribution of fimbrial and medial forebrain bundle afferents to the septum (Raisman, 1969*a*).

Under normal circumstances (i.e. as a result of the normal developmental processes), both fimbrial and medial forebrain bundle afferents terminate in the dendritic field (on shafts and spines), but only medial forebrain bundle afferents terminate on cell bodies. We made a lesion of the medial forebrain bundle, thus deafferenting a substantial proportion of axosomatic sites, and allowed a survival of 2–3 months, at which time all degeneration has been completely removed (Raisman, 1969*b*). A second lesion was then made in the ipsilateral fimbria and the distribution of degenerating terminals was examined by electron microscopy 2–3 days after the second lesion (i.e. the first, long-term lesion was used to deafferent postsynaptic sites, the second, short-term lesion was used to provide a marker of terminals belonging to the second severed pathway).

Under these circumstances we found a large number of electron-dense degenerating terminals on cell bodies. Thus, as a result of prior deafferentation of axosomatic sites, the fimbrial axons in the septum are induced to reinnervate those sites, over-riding whatever morphogenetic constraints so effectively deny these sites to them in normal development.

(5) *Deafferentation-induced synaptogenesis, although leading to abnormal connections, is not a random process, but involves a hierarchy of preferences*

The observation of the formation of abnormal hippocampal projections to axosomatic sites in the septal nuclei might suggest that deafferentation is such a powerful local stimulus as to over-ride all pattern-forming factors, resulting in a sort of free-for-all, random synaptogenesis. That this is by no means the case is shown by observations in the dorso-lateral crescent of the lateral septal nucleus This area was chosen because it receives a bilateral input from the hippocampus

with 44.5% of its synapses from the ipsilateral fimbria and 24.5% from the contralateral fimbria (Field *et al.* 1980; Field and Raisman, 1983).

If one fimbria is destroyed, and a survival time of 2–3 months allowed (when all the electron-dense degeneration has been removed), and then a second lesion made in the remaining fimbria, we found that 2–3 days after the second lesion, the degeneration (marking the terminals of the second fimbria) had risen to 69%, exactly the sum of the two individual fimbrial projections. Degeneration also occurred equally on both sides. Thus, the conclusion was that the removal of one fimbrial input induces the remaining fimbria to take over all its postsynaptic sites. The overall 'strength' of the hippocampo–septal projection is restored, although at the expense of forming an expanded, bilateral projection from the single remaining fimbria.

The non-fimbrial afferent axons giving rise to the 31% of non-degenerating synapses do not appear to respond to the deafferentation stimulus. They do, however, have the capacity to respond. We showed this by simply increasing the survival time after the second fimbrial lesion (or after a simultaneous bilateral fimbrial lesion). In these circumstances, the degeneration disappears, and the number of non-degenerating synapses once again returns to normal. Thus, we find that the synaptogenic response of the non-fimbrial axons is suppressed in the presence of surviving fimbrial axons, even though it is fully competent to reinnervate all the denervated postsynaptic sites previously occupied by the axons of *both* fimbrias when both are cut.

We have no explanation of this remarkable hierarchy of preference in deafferentation-induced synaptogenesis. It may be due to some preferential matching of intrinsic specificities of pre- and postsynaptic elements, to spatial or glial arrangements in the septal synaptic fields, or to factors in the presynaptic axons themselves – such as functional activity or the fact that the responding fimbrial axons have been severely 'pruned' by cutting their main, caudally directed commissural branches in their course back in the contralateral fimbria (Swanson *et al.* 1980).

(6) *Deafferentation-induced synaptogenesis is effective over distances in the micrometre range*

One of the properties of the reinnervated septal synaptic fields is the appearance of presynaptic terminals which, in the plane of section, make contact with more than one postsynaptic thickening (Raisman, 1969*b*). We have called these 'double synapses', 'triple synapses', and so on, and measured the strength of the effect by the 'multiple synapse index'. This measure increases progressively during the course of reinnervation. Its strength is proportional to the intensity of reinnervation (i.e. it is less in partial unilateral fimbrial lesions, greater in complete unilateral lesions, and greater still in bilateral lesions). It reaches a stable level when reinnervation is complete.

In fact, the appearance of double synapses was the observation which first led us to the conclusion that reinnervation was occurring. We speculated that the

synaptogenic influence worked over a small range. When a presynaptic terminal degenerated, it induced the formation of new presynaptic terminals in the immediate vicinity, i.e. within around 1 μm distance. As a result, the new configuration showed signs of its mode of formation by retaining two synaptic contacts established by a single, expanded, vesicle-containing presynaptic element – the multiple synapse.

It should be noted at this point that, if synapse induction is *restricted* to the micrometre range of distance, this would imply major limitations in attempting to exploit this effect in the design of any future strategies for repair of injuries of the central nervous system. One situation in which synaptogenesis appears to be able to extend beyond this range is the ipsilateral part of the lateral septal nucleus (i.e. the main body of the nucleus, excluding the dorso-lateral quadrant). This part of the nucleus normally receives only an ipsilateral fimbrial input but, after section of the ipsilateral fimbria, the crossed fimbrial fibres normally confined to the dorso-lateral quadrant expand down into the main body of the nucleus and establish synaptic terminals for up to about 1.5 mm from their original site (Field, 1980). The conclusive interpretation of this observation, however, still depends on full elucidation of the routes taken by the crossed fibres on their way to the dorso-lateral segment.

An interesting observation arising from this study of the spreading of the crossed fimbrial projection was the finding that, even though the adjacent bed nucleus of the stria terminalis is denervated by simultaneous section of the stria terminalis, the extending crossed fimbrial axons will only make synapses as far as the border of the bed nucleus of the stria terminalis (Field, 1980); they will not cross into it, even though it is denervated and there is no visible boundary separating the neuropile from that of the lateral septum. Once again, specificity is strictly maintained even in deafferentation-induced synapse formation.

(7) *Deafferentation-induced synaptogenesis results in abnormal connections, whose functional effects, still largely unexplored, may be useful, neutral or deleterious*

At this point it may be important to make clear that, however orderly and controlled it may be, degeneration-induced synaptogenesis must result in abnormal patterns of neuronal connectivity. The fibres that were cut originally do not regenerate. Even in the case of fimbrio–fimbrial preference, the patterns are quite wrong. We have to assume that the elaborate and finely regulated nature of the ipsilateral and bilateral hippocampo–septal projections resulting from normal development have functional significance. In that case, the effect of a unilateral fimbrial lesion would be to unbalance the system. The selective denervation-induced reinnervation by the crossed fimbria does not make things better, indeed, it makes them worse. The hippocampo–septal imbalance is not corrected, but reinforced.

Currently we have no way of influencing denervation-induced synaptogenesis. It

is an orderly, spontaneous, predictable and robust reaction. Regardless of whether the new synapses are functional in the sense of transmission (which they certainly appear to be in some systems that have been studied, Steward *et al.* 1976), we cannot say whether the synaptogenic process is functionally valuable, neutral or deleterious to the animal as a whole. It may also have functionally different consequences in different systems. Until we have a method of modifying denervation-induced synaptogenesis by preventing it, increasing it or altering its pattern or time course, we cannot comment on its functional value to the animal.

(8) *The nature of the necessary stimulus to the reinnervating presynaptic terminals is still unclear*

While the postsynaptic partners appear to regulate the overall numbers and distribution of synaptic sites, it is still not clear what stimuli are necessary to induce the presumptive presynaptic elements to form synapses. As has been mentioned, 'pruning' (i.e. removal of other parts of the axonal tree) may have an influence. Patterns of neuronal activity may also play a role. In addition, non-neuronal elements (astroglia) are definitely involved.

(9) *Astrocytes act as an intermediary relay of the deafferentation-inducing synaptogenic signal to the reinnervating presynaptic elements*

Astrocytic processes play a major role in deafferentation-induced synaptogenesis after unilateral fimbrial lesions (although possibly less after bilateral lesions, Field and Raisman, 1983). The astrocytic processes in the vicinity of the degenerating terminals swell, encircle the terminals and come to engulf them and break down the debris. The astrocytic processes clearly displace the degenerating terminals and become themselves apposed to the postsynaptic thickenings (Field and Raisman, 1983; Lund and Lund, 1971; Raisman and Field, 1973; Westrum and Black, 1971). It has been customary to call such configurations 'vacated synaptic thickenings', terminology originating at a time when astrocytes were considered of so little importance as not to be worth mentioning. Astrocyte-apposed denervated postsynaptic thickenings are uncommon, transient structures, only appearing in appreciable numbers at the height of the reinnervation process (Raisman and Field, 1973). The astrocyte-apposed synaptic sites are rapidly reinnervated by adjacent, non-degenerating presynaptic terminals.

Little though we yet understand the probably complex and multiple roles of the astrocytes, it seems likely that the synapse-inducing denervation signals, whether arising from the denervated postsynaptic sites or from the degenerating terminals (or both) are transmitted *through* the intermediary of the phagocytic astrocytic processes which engulf the terminals and directly occupy the sites. A minimal series of events would require (a) astrocytic recognition of, and response to, degeneration of the presynaptic element, and (b) astrocytic recognition of the

ingrowing new synaptic terminal (and retraction from the postsynaptic thickening).

(10) *Deafferentation-induced synaptogenesis may mimic a normal developmental process; deafferentation may reveal an ongoing process in the adult, concealed within an overall dynamic equilibrium, and possibly part of a continuous remodelling, based on function*

The intervention of glial processes in the adult brain may be a reflection of a comparable glial role in development (Rakic, 1971). The whole denervation-induced synaptogenic process results in structures indistinguishable from those produced in normal development. This raises the possibility that the denervating lesions serve to reveal, or re-initiate, synaptogenic potential 'left over' from the development process (minus, of course, some of the normal regulatory effects, such as the axosomatic prohibition on hippocampo–septal axons).

A more exciting possibility is that lesion-induced synaptogenesis is only one aspect of adult synaptogenesis. This process may be going on all the time, in relation to experience and learning, driven by patterns of activation. After all, it seems unlikely that evolution should build in such a precisely regulated adult synaptogenic mechanism simply for the eventuality that scientists would one day lesion the rat fimbria. Rather, it is likely that what we are seeing in the lesion situation is not some totally abnormal process, but an exaggerated view of a normal adult process used in the daily life of the organism.

For us, however, the most attractive aspect of adult synaptogenesis is the possibility that it could be exploited for repair of brain and spinal cord injuries. For this purpose we turned to the formation of connections in adult brain when confronted with embryonic transplants, thus artificially re-introducing an element of developing tissue into the situation.

Studies of embryo-to-adult hippocampo–hippocampal transplants

In a series of light and electron microscopic studies of three different types of embryonic transplants into the adult host hippocampus in rats and mice, we have demonstrated the highly selective reinnervation of specific host hippocampal laminae by appropriate types of presynaptic neurones (Raisman and Ebner, 1983, 1985; Zhou *et al.* 1985, 1989).

One clear correlation established by Björklund and co-workers (Björklund and Stenevi, 1984), and clearly confirmed in our own hippocampal studies (Raisman and Ebner, 1983, 1985; Zhou *et al.* 1985, 1989), was that transplants will only reinnervate host terminal fields if the terminal field is denervated of the same type of input as that of the donor tissue.

This raises the question of whether deafferentation-induced synaptogenesis by host fibre systems can prevent synapse formation by embryonic transplant axons. What would happen after transplantation if the denervated host postsynaptic sites were reinnervated as a result of lesion-induced synaptogenesis by existing adult

host presynaptic neurones (as in the septal studies)? Would the number of postsynaptic sites remain fixed, or would hyper-innervation occur? Would lesion-induced synaptogenesis by adult axons be sufficient to prevent embryonic axons from growing in? Or would some kind of hierarchy, or even sharing of postsynaptic sites occur?

In a preliminary study of the reinnervation of entorhinally denervated adult dentate gyrus by embryonic entorhinal transplants, we found evidence that the fixity of numbers of postsynaptic sites is maintained (P. M. Field, C.-F. Zhou and G. Raisman, unpublished results). But there were some unexpected findings in the time course. First, unlike the septal nuclei, the degeneration, affecting a much greater proportion of the synapses (around 90%) than in the septum after unilateral fimbrial lesions, is removed far more slowly. Even at 6 weeks after operation (when the cycle of removal of degeneration and reinnervation is complete in the septum), the entorhinally denervated dentate gyrus remains full of 'late' (i.e. highly degraded) degenerating presynaptic terminals still apposed to their postsynaptic thickenings.

The second new finding was that embryonic entorhinal transplants into such a *pre*-denervated dentate gyrus caused a major fall in degeneration and accompanying restoration of normal synapses, indicating that the transplant axons are forming synapses in the host. Here, then, was something never seen in the adult lesion situation, the presence of the embryonic tissue seemed to accelerate the otherwise indolent phagocytic reaction of the astrocytes. Whether this is due to the neuronal or to the glial elements of the transplants is still not clear.

What is clear is that synapse-counting studies have not come to the end of their ability to reveal new data, and we hope shortly to be reporting further on this experiment.

In a similar type of lesion and transplant study (Zhou *et al.* 1989) we have used the complementary time pattern, that is to transplant a piece of embryonic entorhinal cortex into a non-denervated dentate gyrus (which it will not innervate), and then, after various survival times, to study the effects of subsequent specific denervation (by removal of the host entorhinal area).

The first observation was that up to 1 week after transplantation, the transplants remained fully capable of innervating the host dentate gyrus. Between 1 and 2 weeks this ability decreased progressively, and beyond 2 weeks the donor entorhinal grafts had completely lost the ability to respond to the subsequent host denervation.

This was contrary to expectation. Why should an embryonic transplant lose the ability to mount a synaptogenic response to denervation when adult host tissue never does so? Currently, we can do no more than speculate on this. Possibly the axonal system of the graft matures in such a way that there are no terminals close to the denervated postsynaptic sites, so that when subsequent denervation is performed, the transplant axon terminals are outside the range of the denervation-associated synaptogenic signals. Only a detailed analysis of transplant axon morphology can approach this issue.

Conclusions and forward look

It is perhaps a measure of the success of a new idea that it becomes accepted so completely as to seem trite. But such an abrogation of uncertainty is the death knell of any idea. 'Dictionaries', as the great Chinese lexicographer Matthews (1931) wrote, 'are the graveyards of a language'. We might say that textbooks, and especially the aura of authoritativeness with which they are presented to the mind in training, are the cemeteries of ideas.

The brain, like language, is a living structure. We have not fathomed its logic. Even the simplest assertions in this article can be questioned. Is deafferentation-induced synaptogenesis a general phenomenon? Or is it confined to the rat septum? Do there lurk, behind the dense shrubbery of persuasive verbiage, entirely different, maybe opposite interpretations of exactly the same results? Above all, perhaps, what experiments can be designed to reveal the mechanism of what has been seen? What mechanisms preserve the unassailable fixity of synaptic numbers and distribution? Where resides that mysterious intelligence that governs the extraordinary selective, intelligent behaviour of adult presynaptic axons?

Recent and fascinating experimental work (Mattson *et al.* 1989) shows that the growth cone and its associated exploratory filopodia act as highly sensitive integrators of diverse information. Axons responding to deafferentation may also develop this apparatus, possibly in a miniature form arising from existing axon terminals within range of the denervation stimulus. This, in itself, would be worth trying to demonstrate. In the formation of such growth cones, the axons may also be reflecting other stimuli acting upon them, such as pruning of other collateral branches or activity changes. Such an induced growth cone/filopodial apparatus would be a prime candidate for sensing the presence of nearby denervated postsynaptic sites as well as the denervation signal relayed by reactive, phagocytic astrocytic processes. Moreover, it may sense the presence of other types of responding growth cones, whose characteristics may serve as the signal for the first growth cone either to retract and give way (Ivins and Pittman, 1989) or to proceed and deny the postsynaptic site to the competing axon system, thus establishing the hierarchy of preference that we observe in the septal nuclei.

While there remains an interest in trying to repair damage to the brain and spinal cord, synaptogenesis will remain at the heart of studies, for without synapses there is no function. The application of counting studies to electron microscopy of the rat septum wrote not an epitaph, but a signpost, to a long, uncharted and exciting road.

References

BJÖRKLUND, A. AND STENEVI, U. (1984). Intracerebral neural implants: neuronal replacement and reconstruction of damaged circuitries. *A. Rev. Neurosci.* **7**, 279–308.

FIELD, P. M. (1980). Synapse formation after injury in the adult rat brain: failure of fimbrial axons to reinnervate the bed nucleus of the stria terminalis. *Brain Res.* **189**, 91–101.

FIELD, P. M., COLDHAM, D. E. AND RAISMAN, G. (1980). Synapse formation in the adult rat brain: preferential reinnervation of denervated fimbrial sites by axons of the contralateral fimbria. *Brain Res.* **189**, 103–113.

FIELD, P. M. AND RAISMAN, G. (1983). Relative slowness of heterotypic synaptogenesis in the septal nuclei. *Brain Res.* **272**, 83–99.

HAMLYN, L. H. (1963). An electron microscope study of pyramidal neurons in the Ammon's Horn of the rabbit. *J. Anat., Lond.* **97**, 189–201.

IVINS, J. K. AND PITTMAN, R. N. (1989). Growth cone–growth cone interactions in cultures of rat sympathetic neurons. *Devl Biol.* **135**, 147–157.

LUND, R. D. AND LUND, J. S. (1971). Synaptic adjustment after deafferentation of the superior colliculus of the rat. *Science,* **171**, 804–806.

MATTHEWS, R. H. (1931). *A Chinese–English Dictionary Compiled for the China Inland Mission.* Shanghai: China Inland Mission and Presbyterian Press, 1226pp.

MATTSON, M. P., MURRAIN, M., GUTHRIE, P. B. AND KATER, S. B. (1989). Fibroblast growth factor and glutamate: opposing roles in the generation and degeneration of hippocampal neuroarchitecture. *J. Neurosci.* **9**, 3728–3740.

PALAY, S. L. AND CHAN-PALAY, V. (1974). *Cerebellar Cortex, Cytology and Organization.* New York: Springer Verlag.

PETERS, A., PALAY, S. L. AND WEBSTER, H. DE F. (1976). *The Finė Structure of the Nervous System: the Neurons and Supporting Cells.* Philadelphia: W. B. Saunders. 406 pp.

RAISMAN, G. (1969*a*). A comparison of the mode of termination of the hippocampal and hypothalamic afferents to the septal nuclei as revealed by electron microscopy of degeneration. *Expl Brain Res.* **7**, 317–343.

RAISMAN, G. (1969*b*). Neuronal plasticity in the septal nuclei of the adult rat. *Brain Res.* **14**, 25–48.

RAISMAN, G. (1970). An evaluation of the basic pattern of connections between the limbic system and the hypothalamus. *Am. J. Anat.* **129**, 197–202.

RAISMAN, G. AND EBNER, F. F. (1983). Mossy fibre projections into and out of hippocampal transplants. *Neuroscience.* **9**, 783–801.

RAISMAN, G. AND EBNER, F. F. (1985). Hippocampal transplants demonstrate the ability of the adult brain to receive and produce mossy fibre connections. In *Recent Achievements in Restorative Neurology*, I, *Upper Motor Neuron Functions and Dysfunctions* (ed. J. C. Eccles and M. R. Dimitrijevic), pp. 280–290. Basel: S. Karger.

RAISMAN, G. AND FIELD, P. M. (1973). A quantitative investigation of the development of collateral reinnervation after partial deafferentation of the septal nuclei. *Brain Res.* **50**, 241–264.

RAKIC, P. (1971). Neuron–glia relationship during granule cell migration in developing cerebellar cortex. A Golgi and electron microscopic study in *Macacus rhesus. J. comp. Neurol.* **141**, 283–312.

STEWARD, O., COTMAN, C. AND LYNCH, G. (1976). A quantitative autoradiographic and electrophysiological study of the reinnervation of the dentate gyrus by the contralateral entorhinal cortex following ipsilateral entorhinal lesions. *Brain Res.* **114**, 181–200.

SWANSON, L. W., SAWCHENKO, P. E. AND COWAN, W. M. (1980). Evidence that the commissural, associational and septal projections of the regio inferior of the hippocampus arise from the same neurons. *Brain Res.* **197**, 207–212.

WESTRUM, L. E. AND BLACK, R. G. (1971). Fine structural aspects of the synaptic organization of the spinal trigeminal nucleus (pars interpolaris) of the cat. *Brain Res.* **25**, 265–287.

ZHOU, C.-F., LI, Y. AND RAISMAN, G. (1989). Embryonic entorhinal transplants project selectively to the deafferented entorhinal zone of adult mouse hippocampi, as demonstrated by the use of Thy-1 allelic immunohistochemistry. Effect of timing of transplantation in relation to deafferentation. *Neuroscience* **32**, 349–362.

ZHOU, C.-F., RAISMAN, G. AND MORRIS, R. J. (1985). Specific patterns of fibre outgrowth from transplants to host mice hippocampi, shown immunohistochemically by the use of allelic forms of Thy-1. *Neuroscience* **16**, 819–833.