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Long-term potentiation, cooperativity and Hebb's cell assemblies: a personal history

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The early history of the experimental work leading to the discovery that long-term potentiation (LTP) embodies Hebb's principle of association is described. In addition, the fallacy underlying the sometimes presumed distinction between 'cooperativity' and 'associativity' in the induction of LTP is pointed out.

Keywords: Hebb; cooperativity; associative; kindling; long-term enhancement; cell assembly

Few people, perhaps scientists least of all, are able to agree on present reality. The likelihood of there being much agreement about history, especially among those who participated in it, is thus vanishingly small. And so it was with considerable reservation that I accepted the invitation to write a historical perspective on the early days of LTP research in the laboratory of Graham Goddard in Halifax, Nova Scotia, Canada. Indeed, I would not have accepted, were it not for the fact that much of the excitement about the LTP phenomenon derives from the astonishing prescience of a Canadian psychologist, Donald Hebb, who, some 20 years before the discovery of LTP, had postulated such a phenomenon and had outlined its implications concerning the mechanism of associative memory (Hebb 1949). Few would disagree that Hebb's ideas form the fundamental basis of our increasingly sophisticated understanding of the properties of neural networks, both in the abstract and as implemented in real nervous systems. Hebb also founded a tradition in Canadian universities and elsewhere of attempting to explain the observations and concepts of psychology in terms of physiological observables. Graham Goddard was a 'grand-student' of Hebb, having worked at McGill under Hebb's student Peter Milner, who both introduced inhibitory control into the cell assembly theory (Milner 1957) and later formulated the first hypotheses on the use of oscillations to solve the binding problem. As an undergraduate, several of my teachers similarly traced their intellectual lineage directly back to Hebb. Thus, out of my deep respect for Hebb and his contribution, I feel some obligation to attempt to write this brief, personal history of his influence on the early developments in LTP research in Canada, and on the direction of my own research career then and now.

When I was 16 years old, my father gave me two books to read. One was Penfield's and Roberts' (1959) *Speech and brain mechanisms*, wherein he described his observations of the mnemonic retrieval effects of electrical stimulation on the temporal lobe. Penfield had concluded that 'the hippocampus of the two sides is, in fact, the repository of the ganglionic patterns that preserve the record of the stream of consciousness. If not the repository, then each hippocampus plays an important role in the mechanism of reactivation of that record'. The other book was Hebb's *The organization of behavior*. So I was, in some sense, 'primed' for a research career involving the hippocampus, synaptic plasticity and memory. Later, as a beginning graduate student in Ottawa, I attended a seminar course on memory, conducted by Dan MacIntyre, a former student of Goddard's and a major contributor to the kindling field (Goddard & McIntyre 1969). I confess that my real reason for taking this course was not primarily my interest in memory, but involved a certain other graduate student in the course by the name of Carol Barnes. Carol was then a student of Peter Fried, also a former Goddard student. In any case, for my term project, I undertook to present to the class the recent ideas of David Marr (1969, 1970, 1971), who was perhaps the first to take Hebb's concepts and formalize them mathematically in the context of the known anatomical organization of synaptic circuits in the brain. Little did I anticipate the difficulty of this undertaking. Marr's 'pre-Hopfield' mathematical framework has proven almost impossibly difficult to follow, even by today's specialists in computational theory. Indeed, some have suggested that the mathematics were just plain wrong (Willshaw & Buckingham 1990); but the strength of Marr's early papers was not in the mathematics, but in his fundamental ideas on the properties of associative networks, such as pattern completion and error correction; on the roles of the various cellular elements of real networks, such as modifiable recurrent collateral synapses and inhibitory synapses with 'shunting' effects; and on the basic activity parameters necessary for a network to store associations, such as sparse, orthogonal patterns. All of these concepts are now fundamental components of our understanding of how networks store experiences. Marr's writings on associative memory in the cerebellum, neocortex and hippocampus preceded the formal publication of the discovery of LTP, but he may have caught wind of it, as there is a 'note in proof' on Lømo's early brief report on synaptic facilitation in the dentate gyrus and also, according to Tim Bliss (personal communication), Marr was a friend of Tony Gardner-Medwin.

A few months later, in the summer of 1973, I had the opportunity to attend a three-week summer school on syn-

One contribution of 30 to a Theme Issue 'Long-term potentiation: enhancing neuroscience for 30 years'.

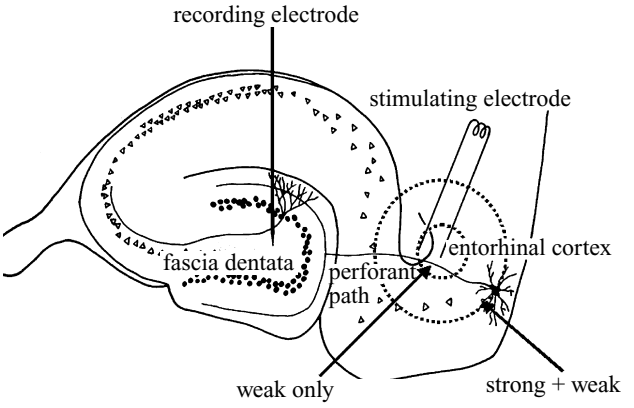

Figure 1. Illustration of the recording and stimulation set-up for the original experiments on cooperativity of coactive inputs in LTP induction. These experiments compared the effects of high-frequency stimulation of a 'weak pathway', that is, a small number of afferent fibres, with the effects on the same pathway when it was stimulated in association with a 'strong pathway', that is, a large number of afferents. This comparison was made using a single electrode. The weak input was activated using a weak stimulus. The combination of strong and weak inputs was achieved by increasing the stimulus intensity during the high-frequency train, and then returning to the weak stimulus for further testing. The same experiment can be carried out with two electrodes, but the principle is the same.

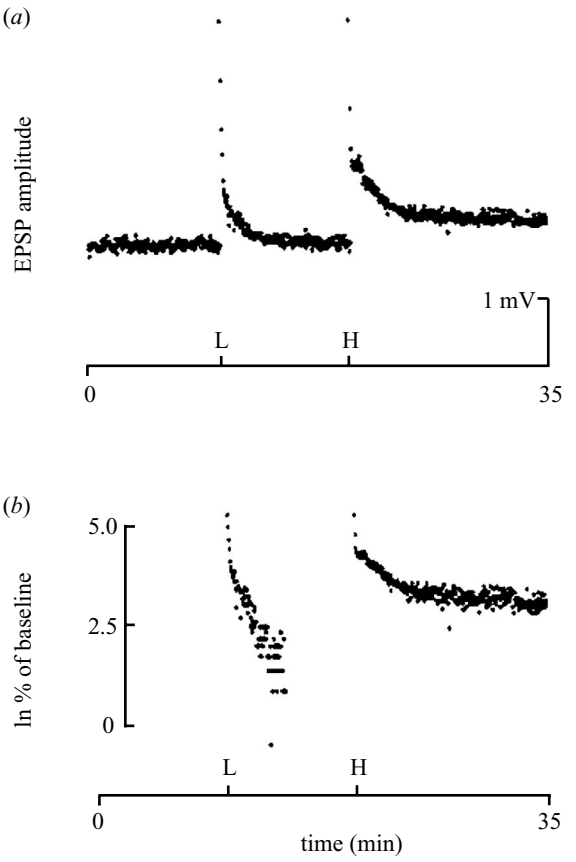

Figure 2. Results of the experiment described in figure 1 are shown in linear and semilogarithmic coordinates in (*a*) and (*b*), respectively. Activating the weak pathway at high frequency (L) produced a large but transient increase in the perforant-path EPSP. The dual exponential decay is characteristic of the processes of 'augmentation' and 'potentiation' which involve an increase in transmitter release probability. Activating the weak pathway in association with a strong one (H) produced the same two fast components and also a third, very slow component, now identified as LTP. This 'cooperativity' was the first indication that LTP embodied Hebb's principle of association.

aptic transmission, held in Erice, Sicily. The meeting was directed by Sir Bernard Katz, and the field was small enough at the time that most of the major players were in attendance as lecturers. One of the informal lectures was given by Terje Lømo, who had just published his work with Tim Bliss on 'long-lasting potentiation' in the dentate gyrus of the hippocampus. Clearly, I was primed to receive this information as the verification of the ideas of both Hebb and Marr. Upon my return to Canada, I had to wait impatiently for photocopies of the Bliss, Lømo and Gardner-Medwin papers from inter-library loan. I read them eagerly when they arrived but was astonished to find that neither Hebb nor Marr was even mentioned. It was clear to me that there was an opportunity for a dissertation project here and I decided I would have to move to London or Oslo to pursue it.

By this time, my relationship with Carol Barnes had become rather more than academic, and we both decided that LTP was the right course for us. Carol's interest was in memory and ageing and it was obvious to her that a loss of LTP functionality might well be an important factor in age-related memory impairment, a conjecture that she has systematically and elegantly verified (see these proceedings). At this point, Peter Fried informed us that there was no need to go abroad to pursue these interests. As it happened, a graduate student in Goddard's laboratory in Halifax, Rob M. Douglas (not to be confused with R. J. Douglas who earlier contributed much to the experimental psychology literature on hippocampus and behaviour) had already replicated the Bliss and Gardner-Medwin experiments, with chronically implanted electrodes in rats (Douglas & Goddard 1975). Graham had spent a sabbatical visit with Tim Bliss at University College London in 1974, where they had attempted, unsuccessfully according to Tim, to induce LTP in rats. Graham had exported the evoked hippocampal field potential method to Halifax where he apparently had had more success, and Douglas was in the process of refining the stimulus parameters and stimulus locations that eventually led to considerably more reliability in producing the phenomenon than had been evident in the 1973 *Journal of Physiology* paper. Douglas had shown that short, high-frequency (200–400 Hz) bursts, mimicking, it was thought, the normal activity of central neurons, was a much more reliable protocol than the extended, relatively low-frequency, long-duration stimuli used by Bliss *et al.*, which had been derived from the earlier 'frequency potentiation' studies of the Oslo group. Douglas had also made very effective use of one of the first laboratory minicomputers, a 12-bit Linc-8, the size of a large refrigerator, with *ca.* 4 kb of memory and a *ca.* 100 kb tape drive. This was programmed in binary assembly code to enable the routine collection and analysis of the large amounts (for the time) of physiological data that would be necessary for a really systematic study of the LTP phenomenon. Later, Graham's laboratory acquired a 16-bit PDP-11 with 32 kilobytes of memory and 1.6-megabyte removable hard

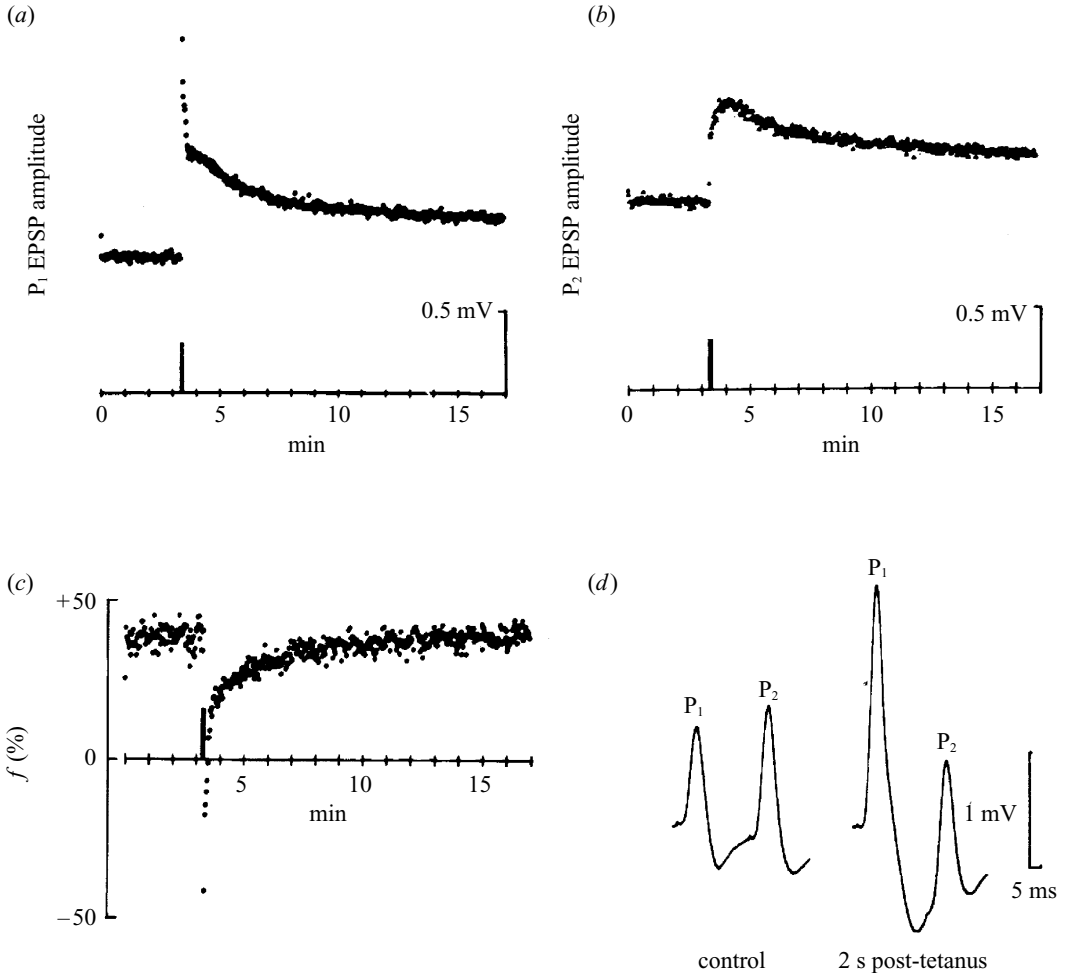

Figure 3. What's in a name? Early evidence that LTP is fundamentally different from the 'potentiation' of neurotransmitter release that had been studied in many types of synapse prior to the discovery of LTP. (Reproduced, with permission, from *J. Physiol.* (*Lond.*) 1982, pp. 249–262, fig. 5.) This study demonstrated the presence in hippocampal synapses of two short-term processes with kinetics identical to the 'augmentation' and 'potentiation' effects (Magleby & Zengel 1975, 1976), which were known to involve increased transmitter release probability. Both processes are elicited in the absence of LTP by weak, high-frequency stimulation, and are especially evident in the lateral perforant path where the resting release probability is inherently low. Augmentation and potentiation decay with exponential time constants of *ca.* 5 and 90 s, respectively. When probed using pairs of stimuli at intervals (25 ms) much shorter than their decay time-constants, an increase in synaptic depression is observed during both processes, in proportion to the elevation in absolute EPSP magnitude. The same effect is observed when transmitter release probability is increased by other means such as elevated calcium ion in the bath. When the same stimulus train is delivered at a higher intensity that produces a lasting enhancement of the EPSP (i.e. LTP), and the result is again probed using paired stimuli, there is an increase in relative depression of the second response of each pair; however, this depression disappears with the same time-course as when there is no long-term change, even though there may be a substantial persistent enhancement of the EPSP. These results showed that LTP is not long-term 'potentiation', but some other process.

disk storage packs, which seemed to us a miracle. In addition to his early direct contributions to improving LTP reliability and to understanding the postsynaptic nature of the locus of LTP induction (Douglas & Goddard 1982), Rob contributed indirectly but enormously to the early LTP research by the selfless sharing of his computer programs with many researchers in the field (myself and Tim Bliss to mention two) at a time when it was simply not possible to buy an effective data acquisition package for such experiments.

Peter Fried arranged an interview for Carol and me with Graham Goddard, who agreed to accept us into his laboratory. Graham, who, in Hebb's tradition, always encouraged innovative research efforts in his students, agreed, in anticipation of our arrival, to purchase a large cohort of adult and ageing rats for Carol's planned ageing research on LTP. That summer, Carol and I were married and departed the same day for the 1000-mile drive to Halifax.

Dalhousie Psychology in the mid 1970s was an extremely exciting place for a young student of the neurosciences because, within a framework of quantitative experimental psychology provided by people like Vern ('working memory') Honig, it had a focus of interest in synaptic plasticity, from early development to associative learning. To highlight a few individuals: Robert Sutherland, who subsequently became a major contributor to hippocampal learning theory and experimental neuropsychology, was a member of the graduate student cohort, and was trying to condition single neurons to fire using brain stimulation reward. Max Cynader and Donald Mitchell were studying

the role of experience and correlated activity in the early development of the visual system. M. Yoon was developing increasingly sophisticated experimental models to elucidate retino-tectal innervation. Ian Meinertzhagen was doing exquisitely detailed anatomical studies on the development of the insect visual system. Some early work in computer modelling of neural networks was also in progress. And Lynn Nadel was there as a visiting lecturer, putting the finishing touches on a long-promised volume with John O'Keefe on the 'Hippocampus as a cognitive map' (O'Keefe & Nadel 1978) that, whatever the final analysis reveals, would revolutionize research into the way the hippocampus processes information. Lynn had been a graduate student in Hebb's department at McGill, and frequently contributed to deep and lofty discussions about cell assemblies and associative memory at the local watering hole.

Part of Goddard's reason for accepting me as a graduate student was that I had had considerable experience in electron microscopy. Goddard had earlier discovered the 'kindling phenomenon', through which repeated daily electrical stimulation of certain brain structures eventually led to electrical after-discharge and behavioural seizures (Goddard 1967). Kindling was, and is, a powerful experimental model for epilepsy research; but Goddard's main interest was in memory, not epilepsy. He saw in the kindling effect, and in epilepsy in general, 'the brain's mechanism for memory gone awry'. During the 5-year period prior to my arrival in Halifax, Graham hade been using electron microscopy in the attempt to find evidence for the 'growth process', possibly the increase in the area of synaptic contact synapse, that Hebb had postulated would underlie the associative mechanism. Goddard's 5-year study attempting to find kindling-related synaptic structural changes in the amygdala had come to nothing. Even if structural changes had been the basis of LTP, the amygdala is simply too complex and diverse a structure, and the EM methods of the day were simply too imprecise and time consuming to yield statistically reliable results in any reasonable period of time. Graham set me the task of using the LTP phenomenon in the dense, homogeneous and monosynaptic connections of the perforant path in the dentate gyrus, to look for Hebb's growth process following LTP.

I was naive enough to the pitfalls and arduousness of such an EM analysis that I agreed to undertake Graham's assignment. I was not so naive, however, as to believe that it was likely, even in the nearly ideal experimental system provided by the perforant path–dentate gyrus, that the LTP derived from stimulation at a single site would alter enough of the relevant synapses to make the needle emerge from the haystack. I knew from the anatomical studies of Hjorth-Simonsen (1972) and Steward (1976) that the perforant-path projection to the dorsal hippocampus arose from a large area of entorhinal cortex, encompassing both its medial and lateral subdivisions, and that only a rather small proportion of the total synaptic population was likely to be activated from one stimulus location. I thought that I might increase my odds of success if I could sequentially induce LTP from many sites across the axis of this projection pathway. Because LTP was, by definition, long lasting, the effects from one site would persist as I systematically moved the stimulating electrode in order to include most of the inputs. Only then would I perfuse the animal and prepare the tissue for electron microscopy. But was the entire entorhinal projection capable of exhibiting LTP? This was not yet known, and had to be investigated first. However, with the high current strengths (300–500 μA) then in standard use to evoke large field EPSP and population spike responses in the dentate gyrus, it would be hard to know how far the stimulus field extended, and therefore hard to know which fibres were responsible for any observed LTP. I decided to do an exploratory study using relatively weak stimulation (*ca.* 50 μA), set well below the threshold for evoking a population spike, and systematically to map the mediolateral axis of the perforant path for LTP expression. My first preparation was a complete failure. The brief bursts that previously had induced LTP hardly left any trace at all, certainly nothing lasting. In order to preserve the improved spatial resolution of using low-intensity stimuli, I decided to try trains of longer duration: 100–200 pulses at 200 Hz. Such stimulation was often known to induce seizures when delivered at high intensity, but I thought that since there would be less postsynaptic discharge, there might be less risk of seizures. I was rather astounded to find that this stimulation indeed induced rather large increments in synaptic efficacy, particularly in the lateral parts of the entorhinal system, but it was transient, decaying smoothly back to baseline in a few minutes. Perhaps LTP was not always long-term. Something about the shape of the decay function reminded me of a recent series of papers by Magleby & Zengel (1975, 1976) on synaptic plasticity in the neuromuscular junction. There was an initial fast decay, followed by a slower one, which, when plotted semilogarithmically, yielded two components that looked very much like what the latter authors had termed 'Augmentation' (3–5 s) and 'Potentiation' which lasted a few minutes and had been described in the early literature as 'post-tetanic' (PTP). Perhaps LTP was merely long-lasting PTP? This was rather disheartening to me, as I knew that these phenomena were entirely presynaptic in their origin, and hence could not embody Hebb's rule. This caused me to begin to think critically of what was the essence of Hebb's idea. It was that a weak synapse could only potentiate if it was activated while the postsynaptic cell was firing. To get the postsynaptic cell to fire in the first place there must be either a coactive strong synapse, or the equivalent, a lot of coactive weaker synapses (which was what Hebb had assumed was typically the case). In my experimental set-up at the time, I only had room to get one stimulating electrode in the perforant path. How could I test the effects on a weak input of coactivating it with a strong (i.e. more numerous) input? Clearly, the way to do this was to use low-intensity test pulses, increase the intensity during the high frequency so as to recruit a much larger number of fibres in addition to the test set, and then revert to the low-intensity stimulation for subsequent testing (see figure 1 and figure 2). I would still be activating the test fibres, and would therefore accomplish the goal of activating a weak and a strong input together at high frequency. Sure enough, the first time I tried it, I observed robust LTP, riding on top of which was apparently the short-lasting 'Augmentation and Potentiation', which was the only effect of stimulating the weak pathway alone.

Hebb had also emphasized that his proposal implied the 'association of two afferent fibres of the same order—in principle a sensori-sensory association', and not just a linear association. By this time, we had realized that the medial and lateral components of the perforant path were separate fibre systems with different sources of input, different biochemistry and different synaptic physiology (McNaughton & Barnes 1977). Could the two pathways, which were both presynaptic to the dentate gyrus granule cells and hence, from that perspective, of the same 'order', cooperate with one another to induce LTP? The answer was clearly yes. Not only could both pathways exhibit LTP on their own, if enough fibres were coactivated, but at low stimulus strength which induced no LTP in either pathway activated by itself, robust LTP occurred when they were coactive. Clearly, the factors underlying the induction of LTP in some way embodied Hebb's associative principle: exactly how would not become clear until several years later, when the properties of the *N*-methyl-D-aspartate receptor and its role in LTP induction were discovered (Collingridge & Kehl 1983; Harris & Ganong 1984).

In preparing these results for publication (McNaughton & Douglas 1978), I made two tactical errors in my choice of vocabulary. The first error was in my choice of the word 'cooperativity'. In subsequent years, several groups repeated almost the identical experiments I described, but used two separate stimulating electrodes to induce their 'strong' and 'weak' synaptic inputs rather than varying the stimulus intensity at a single electrode. They called the resulting LTP 'associative' as though there were some logical distinction pertaining to how the weak and strong inputs had been activated. The misconception that 'cooperativity' and 'associativity' somehow relate to different phenomena has, unfortunately, persisted in the minds of some, and has been a source of considerable confusion to newcomers to the field. The second tactical error was to attempt to change the name 'long-term potentiation' to 'long-term enhancement'. There was, I thought, a very good reason to do so. The cooperativity/associativity effect clearly suggested that 'LTE' was not 'LTP', if by potentiation one was referring to the phenomenon defined as 'potentiation' by the neuromuscular and spinal cord physiologists. Moreover, I had also provided indirect, but reasonably compelling, evidence (see McNaughton 1982) that hippocampal 'potentiation' as well as 'augmentation' involved an increased transmitter release probability, as had been shown at the neuromuscular junction, whereas 'enhancement' did not (see figure 3). Thus 'enhancement' and 'potentiation' were clearly separate mechanisms and I felt it would be useful to the field to keep the distinction clear. As it turned out, the ease with which an acronym 'trips off the tongue' was considered more important. Perhaps so.

There is a final anecdote that is of some historical interest. Donald Hebb was born in a small Nova Scotia town not far from Dalhousie University. He retired there about the time that the cooperativity studies were nearing completion. He was awarded an emeritus professorship in our department and given a small office adjacent to the front door, with a large, black leather, easy-chair. He was there frequently, and his door was always open. After I was convinced that our experiments adequately corroborated his 'neurophysiological postulate' I took the data to his office and explained them to him. He listened carefully and politely, and made a few helpful suggestions, but finally asked why there was so much excitement about this particular part of his theory. The basic idea was, he said, an old one, dating at least to Lorente de No, and the principle was obvious to anyone who had considered the principles of associative learning. Something like cooperativity *had* to be present in the nervous system, there was no other plausible means of association. His suggestion to me was that I would have a much more interesting career if I focused on his cell assembly and phase sequence concepts. I think that he was definitely correct on that score, and his advice was also partly responsible for the fact that the over-ambitious electron microscopy project that was indirectly responsible for the initial confirmation of his neurophysiological postulate was quietly dropped.

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GLOSSARY

EM: electron microscopic
EPSP: excitatory postsynaptic potential
LTE: long-term enhancement
LTP: long-term potentiation
PTP: post-tetanic potentiation