Long-term potentiation in the Eocene

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The first ten years of long-term potentiation (LTP) research are reviewed. Surprisingly, given the intensity of current interest, the discovery paper did not trigger a wave of follow-on experiments. Despite this, the initial work laid out what ultimately became standard questions and paradigms. The application of the then still novel hippocampal slice technique oriented LTP towards basic neuroscience, perhaps somewhat at the cost of lesser attention to its functional significance. The use of slices led to the discovery of the events that trigger the formation of LTP and provided some first clues about its extraordinary persistence. Signs of the intense controversy over the nature of LTP expression (release vs receptors) emerged towards the end of the first decade of work. What appears to be lacking in the literature of that time is a widespread concern about LTP and memory. This may reflect a somewhat different attitude that neurobiologists then had towards memory research and a perceived need to integrate the new potentiation phenomenon into the web of established science before advancing extended arguments about its contributions to behaviour.

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1. MOTIVATIONS AND DIVERSIONS

My interest in LTP grew out of work on anatomical plasticity in adult hippocampus. Just before the appearance of the paper by Bliss & Lømo (1973), we had discovered that removal of the primary inputs to the dentate gyrus causes the remaining afferents to grow new synapses. How long this sprouting took was unclear; it started 5 days after lesions but then proceeded quickly, leaving open the possibility that contact formation might be fast enough to be involved in some stage of memory encoding. I remember reading the two LTP papers and wondering if this was physiologically induced anatomical plasticity. After all, the most startling feature of the new phenomenon was its longevity; something that I then (and now) had difficulty imagining could be achieved without structural changes. Given that we were already conducting electrophysiological studies of sprouting in hippocampus, it did not seem too great a step to search for those changes. However, a second paper derailed this plan even before the first electrode was lowered.

Within days of encountering LTP, I came across one of the papers by Yamamoto & McIlwain (1966) on hippocampal slices. Their results seemed astonishing because for whatever bizarre superstition (sanctity of the brain?), I simply couldn't picture slices of adult brain retaining their *in vivo* physiological properties. However, if this was the case, and the published results were certainly impressive, then it seemed that a revolution was at hand. Seen parochially, it meant that we would not have to search for a small population of synapses in a very large place but instead could stimulate multiple fibre populations converging on a defined dendritic location. (I belatedly real-

ized that 'defined' means one thing at the light microscopic level and something altogether different in the electron microscope.) More generally, however, it was obvious that slices would vastly simplify electrophysiological studies and thereby allow for an unprecedented integration within neuroscience. Slices, in other words, seemed destined to complete the arrival of the hippocampus as the standard model for the cortical telencephalon and thereby allow for happy mingling of the tribes of brain scientists. Given all of this, our laboratory took a sharp turn towards this *in vitro* 'new dawn'.

Why a research group having no experience with culture methods would decide to launch slice experiments without first visiting McIlwain or Yamamoto is lost to memory. This is probably active repression because we had a truly horrible time with it. I remember at one point suggesting that we should move the physiology equipment into the animal colony where an oath would be taken that no one would leave until the slices lived or the rodent population was depleted. My colleagues (chiefly Sam Deadwyler and Tom Dunwiddie) took a second to be sure that this was in fact a joke. Eventually it became clear that we had seen complexity where there was only simplicity, after which slices became the routine business that they are today. Then it was on to LTP and physiologically induced sprouting.

Well, not quite. As the anatomical studies drew near, it seemed only prudent to pause and better define the new potentiation effect. I was surprised that there had not been a rush of papers doing exactly this. In fact, other than a fine replication from Graham Goddard (Douglas & Goddard 1975) and a report of potentiation in slices (and field CA1) (Schwartzkroin & Wester 1975), a strange sort of silence followed the discovery paper. Some possible reasons for this are noted later. Given this state of affairs, we thought it essential to at least confirm that the effect was present outside the dentate gyrus, because that struc-

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

ture, though the object of Bliss' attentions, is peculiar beyond all measure. If LTP was to be a substrate of memory, then we had to be sure that it would show up in more appropriate parts of brain. Then there was the question of whether we should be looking for changes in terminals or spines. Bliss *et al.* (1973) had provided good evidence that the potentiation effect is synapse specific but more could not be said on the basis of conventional physiological results alone. In addition, ahead of everything else, we had to know if it could be routinely obtained in our slice preparations. The first studies revealed LTP to be as robust in slices as in brain, and as prominent in field CA1 as in the dentate gyrus. Regarding location, one would assume that increases in transmitter receptors would amplify the response of the affected dendrites to applied transmitter. Testing this prediction produced a 'wrong' result—the only reliable change accompanying potentiation was a *depression* in the response to glutamate (Lynch *et al.* 1976, 1977). Some time later, Graham Collingridge carried out a much better version of this experiment and found that increased responses do appear, but only after considerable delays (Davies *et al.* 1989). With all that has been learned about glutamate receptors in the past few years, it might be a good idea to revisit this issue.

The depression effect led to systematic investigations of stimulation conditions and the idea of applying a fixed number of pulses at different frequencies. It transpired that high-frequency bursts induce potentiation whereas low-frequency trains cause depression (Dunwiddie & Lynch 1978). Thus, it was the in-between stimulation originally used to elicit LTP that reduced the responses to glutamate and heterosynaptic inputs. I suspect that the same low-frequency effect that was an annoyance in 1978 turned up years later as the much-discussed long-term depression. But the question *du jour* in the earlier period concerned the extent to which LTP was a laboratory artefact or, alternatively, part of the normal life of brain. Insight into this took years to arrive and then from an unlikely source. One day, while trying to make rats believe that electrical stimulation of the lateral olfactory tract was an odour, it suddenly became apparent to Ursula Staubli and myself that we were using completely arbitrary activation patterns. In this case it was a small matter to switch to more naturalistic stimulation (the sniffing pattern) and with this electric odours were born (Roman *et al.* 1987). As I walked away from this rather unlikely discovery, a collision with John Larson brought the realization that sniffing and hippocampal theta are just about the same thing. Within minutes the electric odour pattern, now recast as theta burst stimulation, was producing robust LTP in slices (Larson & Lynch 1986). Recognizing, as confirmed in subsequent experiments, that LTP has a special relationship with the most characteristic of hippocampal rhythms provided reassurance that it had correspondences in the real world.

Returning to 1977, with slices and appropriate stimulation parameters in hand, the search for the anatomical changes hypothesized to stabilize LTP was finally ready to begin. This project turned into a neuroscience equivalent of trench warfare, featuring on the one side an almost intractable problem and on the other a group of researchers driven mad by the idea that a result was just out of reach. Kevin Lee, the graduate student saddled with

the project, assembled a team of undergraduate assistants, many of whom became casualties to the endless piles of electron micrographs. The project dragged on for so long that it became the subject of a scholarly book describing the horrors of frontline neurobiology (M. Lynch 1985 (no relation)).

Finally, evidence for anatomical modifications was obtained (Lee *et al.* 1980) and replicated by a separate group (Chang & Greenough 1984); since then there have been numerous studies demonstrating that changes in synaptic morphology accompany LTP. I still think of these as the most likely explanation for the stability of the potentiation effect, perhaps in the form developed a couple of years later in collaboration with Michel Baudry (Lynch & Baudry 1984). However, it has to be admitted that no one, at least to my knowledge, has yet shown that blocking the morphological adjustments causes LTP to lose its stability and simply decay away.

2. EARLY STUDIES ON THE GENESIS OF LONG-TERM POTENTIATION

Even as we were beginning the structural studies, the group was being pulled ever more deeply into the puzzle of LTP's origins. It seemed best to break this large question into more digestible induction, expression and stabilization stages. Regarding the first of these, calcium was the only outside-in signalling device we knew of and indeed relatively small changes in its concentration proved to have large effects on the production of LTP (Dunwiddie & Lynch 1979). Shortly after this Phil Schwartzkroin reported on the use of intracellular applications of chelating agents to test if calcium is responsible for the after-hyperpolarization that follows bursts of discharges (Schwartzkroin & Stafstrom 1980). We eagerly adopted this technique and were delighted to find that buffering intracellular calcium did indeed completely block LTP induction (Lynch *et al.* 1983). At about the same time, Collingridge and McLennan discovered that antagonists of NMDA-type glutamate receptors suppress LTP (Collingridge *et al.* 1983); when these receptors were found to admit calcium into the cell (Nowak *et al.* 1984), a neat story for the initial event in LTP production was complete. As a bonus, it turned out that the theta pattern, by a clever piece of cellular engineering, unblocks NMDA-receptors, a result that linked the induction step to rhythms occurring in real animals doing real learning. So within approximately 10 years the search for triggering events yielded both a standard model that has aged well and a satisfying amount of new biology.

The effort to identify events that follow the calcium influx got off to a fine start. Wilhelm Gispen visited the laboratory for a few weeks, ruled *ex cathedra* that LTP involves kinases, and together with Mike Browning quickly obtained evidence that high-frequency stimulation does indeed result in phosphorylation of synaptic proteins (Browning *et al.* 1979). Needless to say, I have since always envied that man's energy. Conversations with the newly arrived Michel Baudry led to a much more exotic candidate that seemed particularly appropriate for bridging the gap between calcium influx and long-lasting changes. This was calpain, a calcium-activated protease known from non-neural tissue to cleave cytoskeletal pro-

teins. All of this seemed like genuine progress until the list of things that block LTP began to grow at an alarming rate. Soon, it became evident that LTP will not appear unless a great deal of normally present neuronal chemistry is, in fact, present. Despite all of that, calpain still holds a central spot in my ideas about LTP and recent work from various groups allows me to believe that this is more than simple nostalgia.

Finding the changes that express LTP also proved to be slow going, mainly because Baudry and I could not think of a way to distinguish increased release from more receptors. Bliss and colleagues had begun publishing papers showing that high-frequency afferent stimulation increases release but I could not go along with this because the trigger was clearly located postsynaptically. Why, other than sheer perversity, would induction be in one place and expression in another? Of course, what was really needed was an experimentally tractable prediction that distinguished the pre- and postsynaptic hypotheses. Somewhat later it occurred to me that the colocalization of two types of glutamate receptor in the hippocampal synapses provided for such a test: excluding special assumptions, increased release should result in almost equivalent changes in the currents mediated by the two groups of receptors, whereas postsynaptic changes *limited to one of the two groups of receptors* would not. Two versions of this experiment confirmed the postsynaptic prediction (Muller *et al.* 1988). Happily, Roger Nicholl had the same idea, did the same types of experiment and obtained the same result (Kauer *et al.* 1988). Thus, by the late 1980s, it seemed that expression had been narrowed down to a change in the number or operating properties of AMPA-type glutamate receptors. However, the release hypothesis soon came roaring back, this time in the form of studies using variants of quantal analysis. The resultant battle is certainly deserving of its own sociological analysis but takes us too close to the present for this review. Suffice it to say that today, judging from titles in the literature, most investigators agree with the minimal statement that changes in glutamate receptors are a major component of LTP expression, at least for the time-period measured in typical slice experiments.

Stabilization, the third and, to me, most fascinating of the questions from the late 1970s, proved to be even more resistant to analysis than expression. The structural hypothesis satisfied many of the constraints imposed by LTP phenomenology, most notably extreme duration, but called for spine chemistries that could rapidly reorganize and then restabilize synaptic anatomy. Calpain might satisfy the break-up requirement but we had no idea about how new synaptic architectures could be cemented in place. Indeed, it took almost 10 years for a suitable candidate to come along. Thinking back to those times I realize, with some irritation, that I overlooked an important clue from my own laboratory about stabilization. This was a result showing that LTP can be fully reversed by low-frequency stimulation if the stimulation is delivered immediately after induction (Barrionuevo *et al.* 1980). This apparently fell right into an intellectual blind spot. I simply did not realize what was obvious: LTP must have a consolidation period during which it becomes resistant to disruption and is converted into a persistent form. Had that point been developed in 1980, we would have realized

much sooner that at least one aspect of stabilization occurs over a time-course that excludes a vast array of candidate mechanisms.

3. LONG-TERM POTENTIATION AND MEMORY

One of the more curious features of the early literature is the relative lack of discussion about LTP's potential significance for ideas about memory. An early paper of mine sidestepped the subject by using the expression 'behavioural plasticity' in place of 'learning'. All of this seems strange now but only, I think, because we cannot help but impose on the neuroscience of the 1970s the prominence that memory enjoys today. This anachronism distorts the picture. True, the years leading up to Bliss and Lømo's work established a behavioural context that elevated their results from the status of an interesting observation to a major discovery. Most relevant was the intensifying search during the 1960s for the synaptic correlates of learning in various model systems. The idea going back to the nineteenth century, and aggressively argued by J. C. Eccles, that repetitive afferent activity would produce neurophysiological changes lasting for very long periods had been confirmed, probably most clearly in the seminal work of Eric Kandel on simple forms of learning in *Aplysia*. Thompson and Spencer's extremely influential analysis of habituation/sensitization, followed by Thompson's studies of these effects in spinal neurons, led to the general expectation that use-dependent synaptic plasticity would soon be isolated, even in mammals. But those years also saw claims about the neurobiology of memory—for example, the successful transfer of specific memories via injections into naive animals of RNA from trained counterparts—that were, to say the least, controversial. I suspect that mainstream neuroscientists without the behavioural sophistication of Kandel, Thompson *et al.* were affected by all of this and consequently much more conservative than is the case now, when proposing new substrates for memory. This socio-historical hypothesis might be tested with a survey of how often the word 'memory' was used in neurobiological papers with no behavioural components during the 1970s relative to later decades.

The above argument might also help explain the initially slow expansion of LTP research. Other factors would be the time needed for adoption of the hippocampus as a model system and for converting slices into a routine technique. By the mid 1980s, these steps had been taken, the memory controversies had begun to fade, and the explosive growth in LTP research was underway. Adding fuel to this were the above-noted successes in attaching at least some parts of potentiation to recognizable cellular substrates. In the sense of the philosopher Willard Quine, LTP was becoming enmeshed in that network of associations that stretches across all of science (the 'web of science') and thereby was itself becoming a scientific datum. LTP, rather than an isolated physiological effect, was now simply another element of neurobiology, albeit one with several properties suggestive of an encoding device. Finally, the identification of cellular substrates brought with it, and indeed was partly based upon, tools for blocking the induction step. Using these in behavioural studies provided what seemed to be a direct way of testing for a contribution of LTP to memory formation. Richard

Morris was the first to see these possibilities and the first to generate experimental evidence linking potentiation to particular instances of memory (Morris *et al.* 1986). With this, LTP crossed its Rubicon and set in motion yet another round of debate.

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GLOSSARY

LTP: long-term potentiation
NMDA: N-methyl-D-aspartate