The Historical Significance of the Discovery of Long Term Potentiation: An Overview and Evaluation for Non-Experts

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Abstract

This article evaluates in non-technical language for those not familiar with neuroscience jargon the historical significance of Bliss and Lømo's (1973) landmark discovery of long term potentiation (LTP) by establishing precedent context, describing the finding, and then looking at the subsequent decades of LTP research. To set the LTP discovery in context, the article briefly reviews the precedent theories of synaptic information storage, and the empirical precedents of frequency potentiation, synaptic facilitation, and the identification of the hippocampal area as being memory-related. I then discuss and explain Bliss and Lømo's initial work whereby they found synaptic strengthening that lasted for hours. To better evaluate the importance of their discovery, the article discusses the confirmatory evidence of the decades of LTP research that followed. In this way the article evaluates the replicability, generalizability, and mechanisms behind the phenomena. Perhaps most importantly, I discuss the evidence for LTP being an important mechanism that explains some aspects of learning and memory. The article concludes that at this time Bliss and Lømo's discovery looks to be a profound discovery in the history of science.

Keywords. Long-term potentiation, LTP, memory, neural, synaptic plasticity, hippocampus

I'm haunted by the idea that we don't understand how memory works at the fundamental neural level. If you, the reader, think you know how memory works, then hold that thought, and join me in an exploration of the neuronal basis of memory. We do know a number of things about memory: after thousands of studies one can deduce that memory fades with a particular decay curve over time, that it is reconstructive and malleable, that emotion and some neurotransmitters can enhance memory, and that the amygdala and hippocampal areas seem to be involved within a larger network. But can anyone really say how memory works at a fundamental neuronal level? If no one can with consistent evidence, then that would be astounding given the thousands of studies conducted on memory and given what students learn in college textbooks about memory. Although there are some theories about how memory works, it seems that scientists, if they were to employ adequate scientific skepticism, are not sure how it works. To illustrate this shortfall by way of comparison, we know how computers store memory at the fundamental level, but it may be some time before we have equivalent knowledge about how memory works in humans.

Perhaps the most widely communicated explanation of how memory works at a neuronal level is long-term potentiation (LTP). By looking at the precedents of and follow ups to Lømo and Bliss' (1973) groundbreaking discovery of LTP, I hope to take the reader on a journey of skepticism that provides insight as to what knowledge is still supported and what we are missing. If successful, the history of LTP will provide a scaffold that the reader can use to learn about

memory at a fundamental level of analysis; and reach a greater appreciation of the almost incomprehensible miracle of complex biological memory.

In work that would later lead to the discovery of LTP, Terje Lømo in 1966 discovered that stimulating part of the hippocampal region in a live rabbit resulted in a sustained reverberation in adjacent neurons. This reverberation seemed different from previous work in that the potentiation seemed to decay less rapidly (Lømo, 1966). The theoretical significance of this finding eluded Lømo for several years, though he continued explore empirically in follow-up studies (e.g., Lømo, 1971a, 1971b). It was not until Timothy Bliss joined Lømo that the theoretical significance of the findings was solidified, synthesized, and published. Bliss and Lømo's (1973) landmark article stated the concept of long lasting potentiation, based on their empirical findings of sustained synaptic strengthening in the rabbit hippocampus following a burst of high-frequency stimulation of adjacent neurons. In this article, I trace the precedent theories and empirical work that foreshadowed the LTP discovery, outline the discovery itself, and finally discuss why, despite hundreds of LTP studies, there is still some uncertainty about the neural basis of memory. It is only with this broad approach of discussing the contextual precedents and follow-up research can the discovery of LTP by Bliss and Lømo be fully evaluated. At the end of the article those readers who are not neuroscientists should have a better grasp of whether the discovery of LTP was in fact a major breakthrough in the history of science, or not, and whether we yet understand how memory works at a neuronal level.

Precedent Neural Theories of Memory before the Evidence

To set Lømo and Bliss's identification of LTP in context, it is necessary to outline the context of the precedent theories of memory. In one of the earliest theories that directly foreshadowed theories related to LTP, philosopher Alexander Bain (1872) wrote that for every act of memory there is a "specific grouping or coordination of sensations and movements, by virtue of specific growths in the cell junctions" (p. 91). In a similar vein, later that century American psychologist William James's (1890) explained memory in the following way: "When two elementary brain-processes have been active together or in immediate succession, one of them, on recurring, tends to propagate its excitement into the other" (p. 566). These precedent hypotheses put forward by Bain and James somewhat loosely outlined the idea that neurons that fire together may result in a change in the connectivity between them.

In one of the most influential early neural theories of memory, Spanish neuroanatomist Santiago Ramón y Cajal (shown below in Figure 1) proposed that memory might be stored in a network of neurons via strengthening the connections between neurons (Cajal, 1894). This proposition came about from two developments, both of which involved Cajal. One development was the staining of neurons, using the Golgi stain, which revealed a complex network of interconnected neurons. These stains uncovered enough detail to suggest that neurons were connected via very small gaps called synapses, which Cajal indicated in his theorizing and illustrated in his neural network diagrams. The other development towards the end of the 19th century was the idea that the number of neurons in the adult brain did not increase significantly in number. Because new neuron growth therefore seemed an insufficient explanation of how new memories form, Cajal suggested a theory involving changes in neuron connectivity, manifested in synapse strength.

Figure 1. Following his intricate use of the Golgi stain to reveal intricate networks of neurons, and apparent synapses between neurons, Santiago Ramón y Cajal theorized that memories might be stored across synapses. (Photo: Anon, Clark University, 1899)

Decades later, Donald Hebb (shown below in Figure 2), echoing and extending the ideas that came before, proposed that a network of neural cell assemblies underlie memory, and such neurons may grow new connections as well as undergo changes that their ability to communicate (i.e. changes in the strength of synapses). Hebb (1949) wrote that when one neuron repeatedly triggers another neuron to fire "the axon of the first cell develops synaptic knobs (or enlarges them if they already exist) in contact with the soma of the second cell" (p. 63).

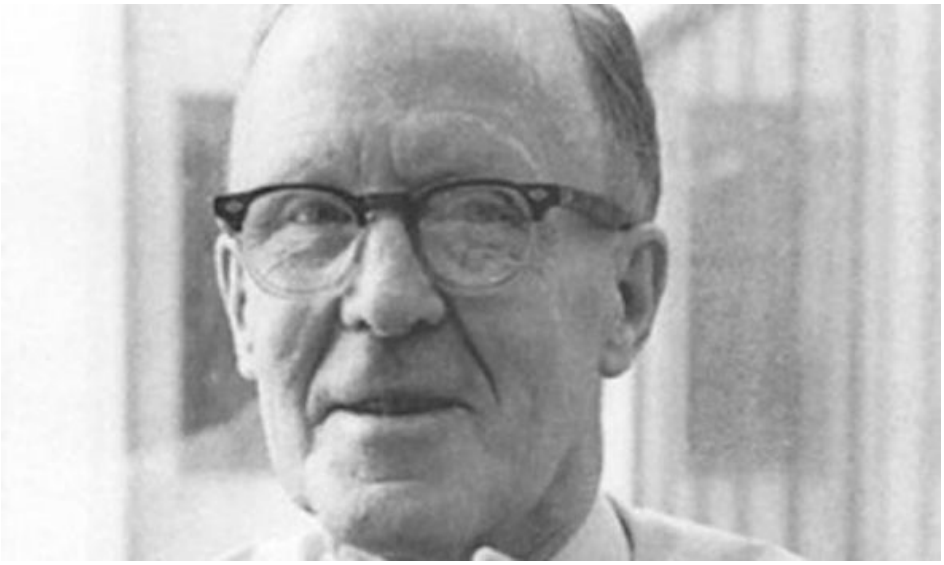

Figure 2. Neuropsychologist Donald Hebb who proposed that neural cell assemblies could be involved in learning through changes in synaptic strength between neurons. (Photo: Original source unknown: used in Rogers, 2017 with label "not-attributed").

This theory that memory could be explained by synaptic strength changes within an internetwork of neurons is often called Hebbian theory, although as noted above, features of this theory were proposed before Hebb by Bain, James, and Cajal. In fact, Hebb (1949) alludes to the point that he was not the first by writing: "The general idea is an old one" (p. 70). However, Hebb's book only managed to credit Cajal once by name (p. 230, without direct citation), and failed to name Bain and James in the context of learning and synapses. However, by comparison to the aforementioned precedents, Hebb's work discussed the theory of cell assemblies and synaptic strength as a basis of learning in greater length and detail. For example, Hebb (1949)

writes: “any two cells or systems of cells that are repeatedly active at the same time will tend to become 'associated', so that activity in one facilitates activity in the other" (p. 70). It was an important synthesis of ideas.

At that time, Hebb did not specify a brain area that might deal with memory. In fact, Hebb theorized that learning may be spread out throughout the cortex. This thinking was perhaps influenced by the work of Karl Lashley, and others, whose decades of research led to Lashley’s conclusion that there is no specific area of the brain that specialized in memory processes (summarized towards the end of the research program in Lashley, 1950). However, the work of Scoville and Milner (1957), which revealed that Lashley’s conclusion was essentially a type II statistical error, and that there is in fact a relatively small area of the brain that seems particularly important in memory processes. The identification of the hippocampal region as a place of special interest for memory is another essential precedent that shaped the later identification of LTP, and is discussed next.

Brenda Milner and the Hippocampal Area

As well as the important theoretical precedents described above, there was also one particularly important empirical finding that shaped LTP research. Scoville and Milner (1957) suggested that a relatively small hippocampal area of the temporal lobe seemed to be crucial in the consolidation of new episodic memories into long term memory. In one patient in particular, H.M., they found that the bilateral removal of the hippocampi and some of the surrounding cortical area (and amygdalae), resulted in a profound anterograde amnesia. H.M. could hold recent episodic events in mind for tens of seconds, but seemed to be unable to hold on to them, such that he could not remember what had just been happening a couple of minutes later. The transfer of memory into long term memory seemed to be completely destroyed. And this large effect came from removing a relatively small volume of brain tissue. This cemented into scientific discourse the idea that the hippocampal area was a structure involved in memory. This inspired researchers in later work to focus their attention on the hippocampus, including the Norwegian neuroscientist Per Anderson and his student, Terje Lømo.

Lømo’s Story: The First Identification of LTP

It was a purely chance meeting with neuroscientist Per Andersen on the streets of Oslo in 1964 that put Terje Lømo (shown below in Figure 3) on the path toward identifying LTP. At that time Lømo was a physician in the Norwegian Navy (Lømo, 2003) and Per Andersen was a professor at the University of Oslo. Andersen told Lømo that he was looking for people to work in his laboratory, and Lømo joined Andersen’s team in 1964. Importantly, Andersen introduced Lømo to ‘frequency potentiation’: the use of repetitive stimulation of a certain frequency that led to a noticeable increase in firing of adjacent neurons. This research had grown out of decades of work by physiologists studying post-tetanic potentiation at the neuromuscular junction in the peripheral nervous system. Andersen’s lab had already found that these rapid volleys of stimulation (called tetanic stimulation) of the perforant pathway of the hippocampus led to the synapses linking to the dentate area of the hippocampus strengthening for a few minutes (published a couple of years after data collection in Andersen, Holmqvist, & Voorhoeve, 1966). This may seem like a similar finding to Lømo’s (1966) subsequent discovery of LTP, but the main difference is the duration of the effect: Lømo’s findings showed a synaptic strength increase that lasted more than 20 minutes, and potentially longer, whereas prior findings found effects for just several minutes. Previous work on post-tetanic potentiation (PTP) that lasted a

few minutes did not provide enough explanatory power as to how learning might be held in the brain for extended periods of time.

Figure 3. Terje Lømo in 2003. Lømo was one of the first researchers to identify a strengthening in synaptic connections that lasted a period of hours, rather than minutes. (Photo credit: Martin Toft, Uniforum, UiO. Reproduced with permission of Martin Toft).

It is interesting that Per Andersen's (2003) account, compared to Bliss and Lømo's (1973) account of the discovery (see also Lømo, 2003), provided a fuller description of the precedent empirical work that occurred before LTP. Anderson (2003) noted that he had found short term potentiation by using high frequency stimulation (Andersen et al., 1966; discussed above) but also pointed out that several others had found something akin to short-term potentiation prior to 1966. For example, Lloyd (1949) reported post-tetanic potentiation (PTP) at spinal synapses that would last up to 7 minutes. Soon after this, Eccles (1953) described how such studies on PTP could perhaps explain learning. Work by Green and Adey (1956) and Gloor, Vera, and Sperti (1964) also described similar findings involving short term facilitation between neurons. In similar research, Kandel and Tauc (1963, 1964) investigated heterosynaptic facilitation, and produced some of the first empirical data of synaptic strength changes in what they proposed was a rudimentary type of learning in the sea slug *Aplysia*. For example, Kandel and Tauc (1964) found increased synaptic strength between paired neurons that persisted for as much as 20 minutes. So the practice of using high frequency pulses to potentiate neurons, and the idea that the resulting 'facilitation' of the synapse could help explain memory, were already known to Andersen, and by extension were absorbed by his graduate student Lømo. What was missing was an account for how relatively short facilitation on synaptic strength could account for learning that lasts longer than a few minutes. This precedent knowledge, combined by the identification of the hippocampal area as being important in memory (Scoville and Milner, 1957; discussed above) laid the groundwork for Lømo's subsequent empirical findings.

Lømo (1966) used the techniques that Andersen had passed on to him: namely he worked on anesthetized rabbits using frequency potentiation. In the initial and subsequent studies from 1966 to 1973 the hippocampal area was accessed by removing some of the rabbit's cortex that lay over the hippocampal area. In the initial investigation, Lømo (1966) observed increases in

amplitude with successive high-frequency stimulations, and subsequent activation of adjacent neurons occurred with less stimulation than they had before. One possible explanation for this was facilitation in the synapses. Lømo also found that the amplitude of the monosynaptic first spike increased to a new maintained potentiated level, and remained there. In this experiment, the pulses were delivered every 7 minutes except for the last two trains, which were separated by 22 minutes. So although the initial investigation was not designed so that it could show hours of potentiation it showed promise of something different from previous studies of the relatively short-lived PTP. According to Lømo (2003), only one slide of that original paper presentation remains of the original Lømo (1966; shown in Lømo, 2003) study, but he soon followed up with a number of studies which were subsequently published in journals.

For example, in one follow up study, Andersen & Lømo (1967) discussed a "possible relationship between frequency potentiation and learning" by hypothesizing that the increase in dentate neuron firing implied "an enhanced efficiency of synaptic transmission outlasts the stimulation by a certain period, from several seconds up to a few minutes. This duration is of the same order of magnitude as that of the post-tetanic potentiation. It is too short to account for the plastic changes in a neuronal circuit that might take place in learning processes of a higher kind. However, if frequency potentiation takes place in a set of neurons constituting a polysynaptic chain, the individual effects may be greatly enhanced" (p. 410). As can be seen here, they were moving closer to finding an effect that was different from previous PTP findings, but still had not reported long term potentiation that lasted hours.

Significantly, Lømo at some point changed his experimental procedure. The disadvantage of reactivating the neurons periodically with high frequency bursts is that you could not find out whether a given effect would last longer than the period between pulses. Crucially, Lømo began to use high frequency bursts only at the start of the experiment to allow for the long term aftereffects to be measured without the periodic interference of additional tetanic bursts. This was a major turning point that allowed for experimental manipulation of the frequency to see if the duration of the effect could be extended. This eventually led to the finding that a single tetanic stimulation of the correct frequency could produce hours of long term potentiation in the adjacent synapses. This finding was most clearly presented in Bliss and Lømo (1973).

Bliss and Lømo Build a Clearer Story of LTP

Building on years of research (Lømo, 1966; Anderson & Lømo, 1967; Lømo, 1971a, 1972b; Bliss & Lømo, 1970), Bliss and Lømo (1973) finally published a full and explicit account of what they called 'long lasting potentiation' (to be later renamed long term potentiation; LTP). To my knowledge, what seemed to set Bliss and Lømo's (1973) article apart from their earlier articles is their change in strategy from using multiple tetanic pulses to using an initial tetanic pulse preceded by and followed by weak probing stimuli, and the subsequent patient monitoring of the long term potentiation for hours. Using this technique, as well as varying the frequency of that single pulse experimentally, is what allowed the researchers to eventually find a frequency and duration that led to a long-term effect over multiple hours. Figure 4 below shows the setup used by Bliss and Lømo (1973) and features the anatomy of the hippocampus, including the perforant pathway (PP) fibers and the adjacent dentate area (AD) interneurons.

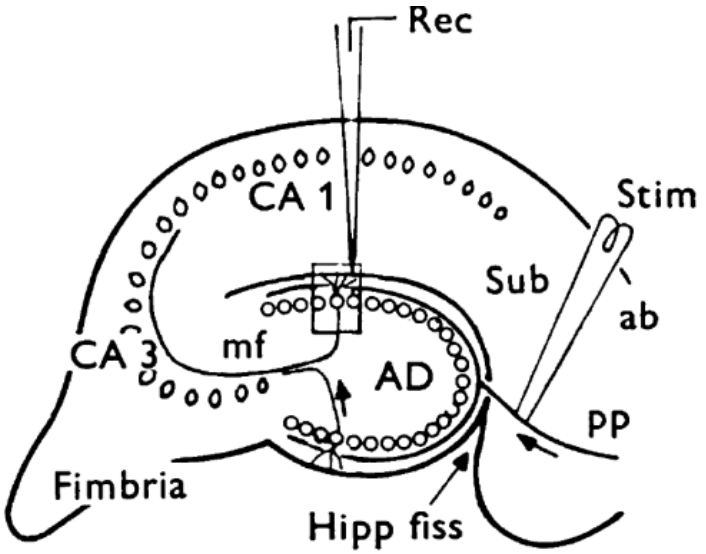

Figure 4. The hippocampus and the set up used by Bliss and Lømo (1973) whereby the perforant pathway (PP) is stimulated ("Stim") with a high-frequency burst, and the recording ("Rec") in the dentate area (AD) reveals a strengthening in the connecting synapses that lasts for hours. Diagram from Bliss and Lømo (1973); reproduced with permission from Dr. Terje Lømo.

Bliss and Lømo (1973) observed that weak (low frequency) stimulation of the perforant pathway corresponded with a baseline low level of activity in the dentate area, but following a high frequency stimulation of the perforant pathway (a tetanic stimulation), subsequent weak stimulations would more easily cross the synapse and maintain a significantly higher rate of firing in the dentate interneurons, compared to before the high frequency pulse. This apparent strengthening on the synaptic connections between perforant path neurons and dentate neurons lasted for hours. This is perhaps easier to visualize in the graph shown below in Figure 5.

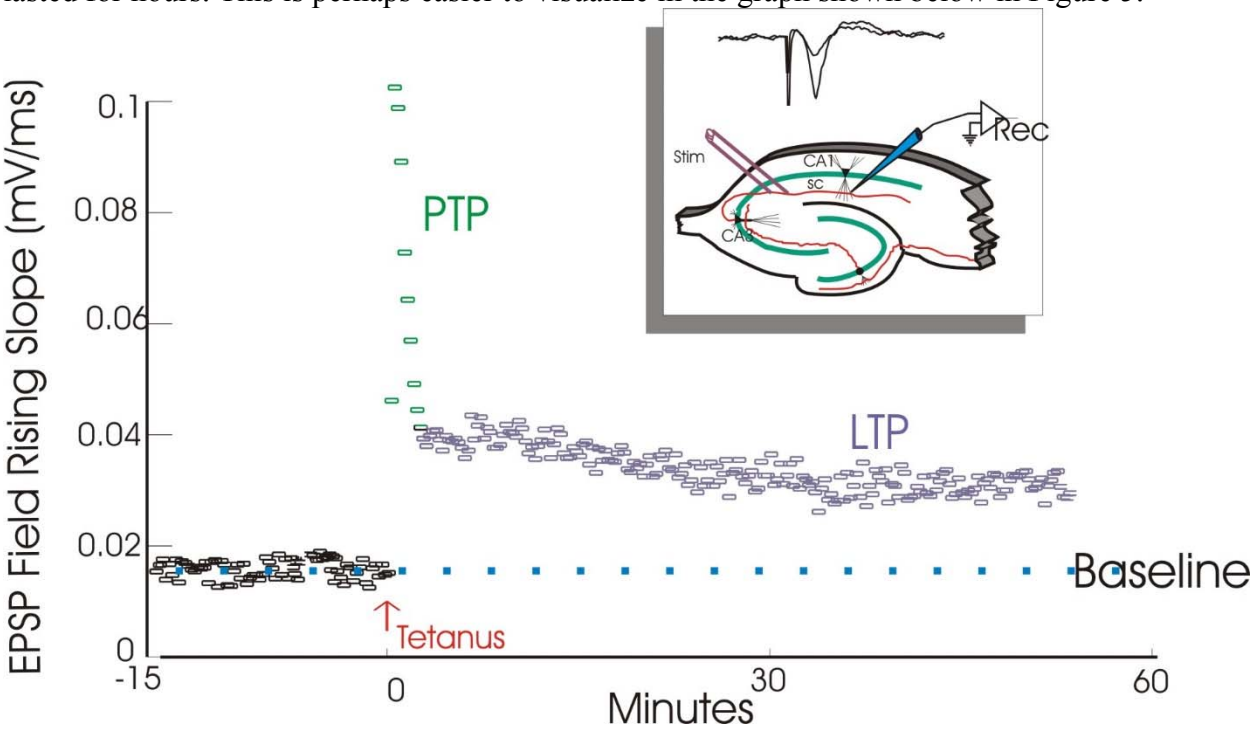

Figure 5. Simplified representation of a demonstration of LTP, showing prolonged synaptic strengthening following a high frequency pulse (tetanic stimulation; red arrow). The test stimulus before and after the tentanus are weaker and not high frequency, yet are able to trigger an

elevated firing of dentate neurons for hours after the high frequency pulse. EPSP means excitatory postsynaptic potential. PTP = post-tetanic potentiation. (Diagram attribution: CC-BY-SA-3.0; http://creativecommons.org/licenses/by-sa/3.0/; reproduced in accordance to Creative Commons attribution guidelines)

The fact that LTP lasted hours in the Bliss and Lømo (1973) seemed like a breakthrough because it promised to be that special neural mechanism that would finally explain learning and information storage. That same year, Bliss & Gardner-Medwin (1973) went a step further to suggest early evidence that one tetanic stimulation could induce LTP in the rabbit dentate area for up to three days. Indeed, in one outlier rabbit, LTP appeared to last for 16 weeks. These increased timescales could hypothetically help explain longer processes of memory such as consolidation and long term information storage. However, there were a number of possible confounds and problems that prevented the conclusion that LTP explained memory at a fundamental level. For example, it could be that the findings don't replicate, or that the electricity from the probe does not mimic neuronal learning as it occurs naturally, or that the mechanisms behind the discovery are insufficient to support the original LTP findings. It is essential, then, to look at significant later LTP studies to better evaluate the historical importance of Bliss and Lømo's discovery. In simple terms, the next sections investigate whether LTP turned out to a well-supported finding, and whether it provides a neural explanation of memory.

Important Later Findings in LTP Research: Did LTP Turn Out to be Well Supported?

I address this question in three ways: discussing replicability, generalizability, and whether the identification of mechanisms (such as the NMDA receptor and related discoveries) further supports the initial Bliss and Lømo (1973) discovery.

Is it a reliable and robust finding? At the time of publication, it was possible that Bliss and Lømo's (1973) findings were a fluke confined to a peculiarity of their equipment and the way the rabbits were prepared. However, follow up studies addressed the replicability of the phenomena and some of the potential confounds. Thousands of LTP experiments were conducted in the decades following Bliss and Lømo's findings (for a focused review, see Nicoll & Roche, 2013), and that fact alone demonstrates the replicability on the finding.

Generalizability. If one considers how LTP has been incorporated into the general theory of synaptic plasticity, it becomes essential that LTP demonstrates generalizability. For example it was initially unknown whether LTP would be found only in anesthetized animals, and perhaps only in some species, and these two potential confounds were somewhat satisfied by LTP studies on *unanaesthetized* rats (e.g. Dragunow et al., 1989; Jeffery, Abraham, Dragunow, & Mason, 1990), rabbits (e.g. Robinson and Reed, 1992), and cats (e.g., Baranyi, Szente, & Woody, 1991). If LTP is used as part of a theory that underlies information storage more generally, then generalizability to other brain regions is an important issue. For example, if LTP is held to account for motor learning or other cortical functions, it would be useful to demonstrate the phenomena not only in the hippocampus, but in higher cortical areas. Indeed, a number of studies have demonstrated LTP in non-hippocampal areas (e.g. in rat cortex: Feldman, 2000; in visual cortex of rats *in vitro*: Kirkwood, Lee, & Bear, 1995; in the rat somatosensory cortex: Feldman, Nicoll, & Malenka, 1999; in the rat spinal cord: Liu & Sandkühler, 1995; and tentative evidence in the human cortex via TMS: Esser et al., 2006). These findings support the idea that LTP could possibly explain a certain type of information storage in neural networks. There are, though, other possible mechanism involved in memory at the neuronal level (e.g., long term depression, LTD).

Does the identification of mechanisms add further weight to the initial discovery? As long as the mechanisms behind LTP were a mystery, some uncertainty as to what was occurring in Lømo and Bliss's original studies remained, and indeed whether they were observing a biologically natural occurrence was a legitimate concern. Without knowing the mechanisms behind LTP, for example, you could not be sure whether LTP generated by the artificial stimulus came about from identifiable natural processes that would occur in animal neurons without artificial stimulation. The subsequent identification of the chemical ions, neurotransmitters, and receptors (NMDA & AMPA) as parts of the mechanism helped allay some of these fears. Although the tetanic stimulus may be artificial, it is not unreasonable given the multitude of studies (for a review of LTP mechanisms see Nicoll & Roche, 2013) that the underlying mechanisms (ions, neurotransmitters, receptors) occur *in vivo* in animals without artificial input. The discovery that LTP seems to be achieved via NMDA and AMPA receptors, and that long term synaptic strengthening requires protein synthesis, and that this synthesis is targeted to relevant synapses only, all serve to enhance the historical importance of Bliss and Lømo's (1973) original findings. An analogy would be that we are more certain that we actually went to the moon if we understand the small-scale step-by-step mechanisms that explain how we got there. Similarly with LTP, we can be surer that it exists as a natural phenomenon if we have an evidence-based explanation of how it is achieved. There is, however, still uncertainty as to the extent that late stage LTP is dependent on protein synthesis (see Sharma, Nargang, & Dickson, 2012; Canal, Chang, Gold, 2007; Routtenberg & Rekart, 2005).

Does LTP Explain Memory?

Does LTP last long enough to explain memory? As mentioned earlier, although Bliss and Lømo (1973) described LTP lasting as much as 10 hours, Bliss and Gardner-Medwin (1973) uncovered some tentative evidence that LTP might last a few months in some circumstances. Indeed, subsequent studies from various research teams confirmed that LTP can last many weeks with some tetanic stimulation patterns (e.g., Racine, Milgram, & Hafner, 1983; Staubli & Lynch 1987; Jeffery et al., 1990; Abraham et al., 1993; see Abraham, 2003 for a review). Over time, LTP studies began to describe early and late types of LTP: early LTP that lasts up to a few hours, and late LTP that lasts for many hours. Interestingly, early LTP seems to use proteins already present in the neuron, whereas late LTP that lasts an especially long time (i.e. LTP3 ~ 25 days; see Abraham et al., 1993) tends to involve new protein synthesis via the neurons' genetic material in the neuron cell body (Krug, Lossner, & Ott, 1984; Otani, Marshall, Tate, Goddard, & Abraham, 1989; Nguyen, Abel, & Kandel, 1994). The take-home message here is there appears to be a type of LTP that lasts long enough to explain information storage processes in the brain that occur over months. Perhaps one of the most vivid demonstrations of this type of late LTP is shown in Figure 6. This graph shows data from Abraham, Greenwood, Logan, Mason-Parker, & Dragunow (2002) in which the LTP following 4 tetanic stimulations lasted for about a year.

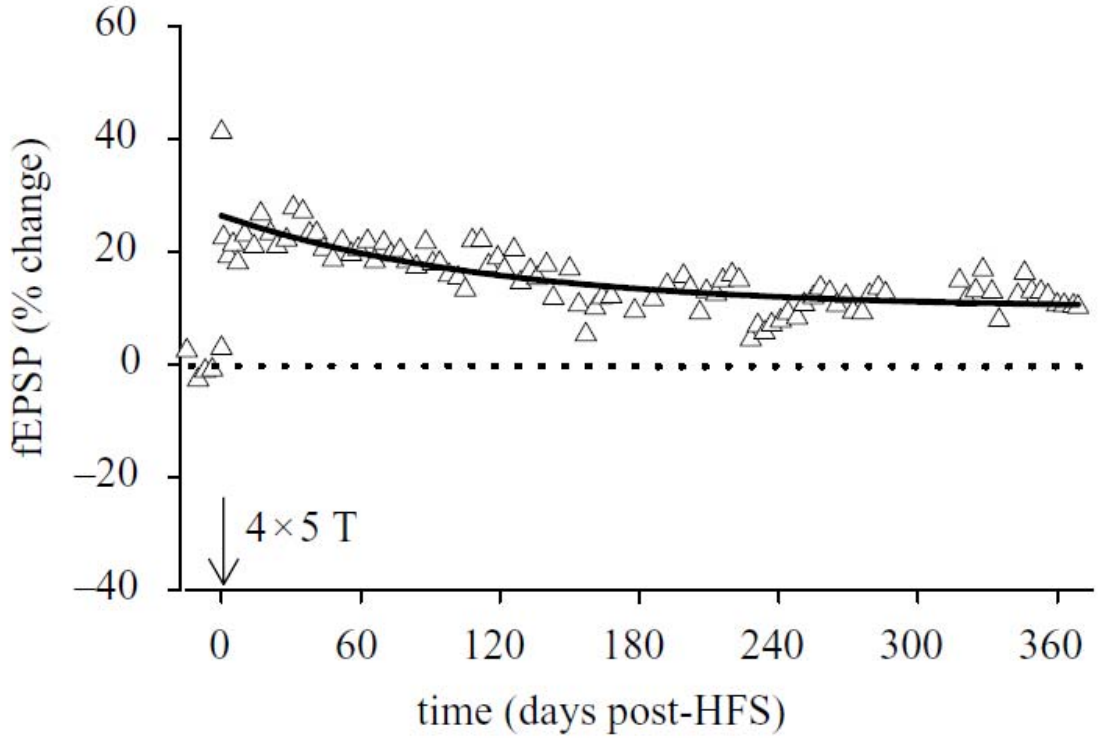

Figure 6. Yearlong persistence of LTP. Data from Abraham et al. (2002) showing the long term potentiation persisting for a period of 365 days. Notice the shape of the decay curve: Abraham (2003; see his Figure 3) describes how the decay curve of both early and late LTP follow a negative exponential curve. In the case of late stage LTP (LTP3, shown here) the decay curve is slight, but still noticeable. Reproduced with permission of Dr. Wickliffe Abraham.

It is uncertain whether long term potentiation can last a number of years, as would be needed to explain long term learning. Such research is constrained by practical matters, such as keeping an animal alive for extended periods of time after part of their skull and cortex is removed with an *in vivo* approach, or keeping excised tissue alive for long enough with an *in vitro* procedure. At the very least, LTP lasting months does give some credence to the possibility that this type of LTP (LTP3, in this case) could be similar to the mechanism behind long term information storage in the brain. If LTP is the general mechanism behind information storage, it should be evident in other brain regions too, and Abraham (2003) states that there are some, but not many, studies of LTP persistence outside the hippocampus. The very slow decay curve shown in Figure 6 perhaps is associated with the slow decline of memory, although it should be pointed out that the LTP was created artificially, and not by a learning event. This last point raises the question as to whether LTP has been shown to arise from an event involving memory in a live animal.

Has LTP been demonstrated following actual learning in a live animal? An additional possible confound, mentioned briefly above, is that the tetanic stimulation of a nerve is somewhat artificial, and that it originates not from learning but from electrical stimulation. The problem is that it does not mimic the natural processes that occur in real instances of learning in natural settings. In effect, what is required to address these concerns is an *in vivo* demonstration of LTP being induced in a live animal following a learning exercise. Indeed, if this is

demonstrated, it vastly enhances the historical importance of Bliss and Lømo's original studies. Some are skeptical that this has yet been achieved with sufficient certainty. However, Whitlock, Heynen, Shuler, and Bear (2006) claimed to have demonstrated LTP in live animals following a learning event. Whitlock et al. (2006) used a one-trail inhibitory avoidance task as their learning event in rats. Specifically, a dark side of the rats' chamber had an electrically charged floor such that the rats would receive shocks if they crossed over to the dark side (cf., Lucas, 1977), and *learn* to avoid that side of the cage. One control group of rats experienced no foot shock in the dark area of the chamber (walk through controls), and another control group was given a shock on the dark side alone and immediately removed such that they would not form a chamber-shock memory association. Whitlock et al. (2006) implanted an 8-electrode recording array into the CA1 area of the rat hippocampus, *in vivo* before the training began. Baseline measurements of the CA1 neuron activity that followed weak stimulation of the Shaffer neurons that synapse at the CA1 neurons. Then the rats in the experimental group were exposed to the inhibitory learning exercise (or controls to walk through or shock only), and then the synaptic strength between the Shaffer and CA1 neurons was again probed. The results seemed to suggest that something that resembled LTP occurred in some rats at just some electrodes some of the time. For example, Figure 7 shows what might be interpreted as an LTP effect in two of the electrodes (color coded as red and orange dots) in a single rat. However, there are a few possible problems with this data: one is that the data for this single rat may have been chosen simply because it looked the best. Another possible problem is that the pattern may not have been discernable without color coding the uppermost two electrodes in colors that sharply contrast with the colors of the non-LTP demonstrating electrodes. One could, for example, look at the dots in totality and conclude they are equally spread around the baseline. Therefore, an interesting question here is: if some electrodes fail to show an LTP response is that evidence against learning inducing LTP? Or is it simply evidence that those electrodes were not near the neurons that did show LTP? If it is the latter, finding LTP in some rats and some electrodes, and not others, could nevertheless be taken as good evidence of a learning-LTP link.

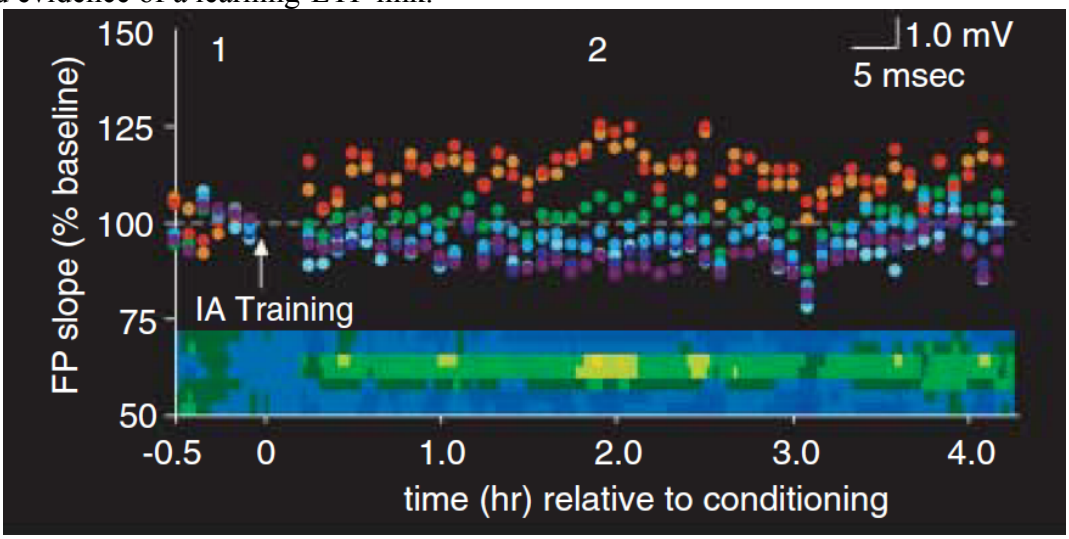

Figure 7. LTP-like pattern of response in the CA1 hippocampal cells lasting four hours in a single rat following an associative learning task involving the avoidance of a foot shock, from Whitlock et al. (2006). Notice the effect is only demonstrated in two electrodes (red and orange dots). Reproduced with permission of Whitlock et al.'s corresponding author Dr. Mark Bear.

Perhaps stronger evidence from Whitlock et al. (2006) is represented in Figure 8, below, which shows that on aggregate rats in the learning condition showed tentative signs of an LTP-like response more often than the other conditions. This is shown in the orange and red colored points on the graphs: these colors highlight a marked elevation of post-synaptic activation over pre-learning baseline. This evidence shows a possible effect across more than one rat, although the results still show a degree of measurement error and noise. Whitlock et al. (2006) would argue that such noise and failures in some rats and some electrodes could be due to the difficulty of placing the electrodes by chance near the CA1 hippocampal neurons that undergo LTP. Notice a crucial comparison with the shock-only group that showed much less deviation from baseline following a shock *without learning*. This rules out the possibility that electricity from the foot shocks is a confound that accounted for the increased potentials in the electrodes. Other controls also show little evidence for a LTP pattern.

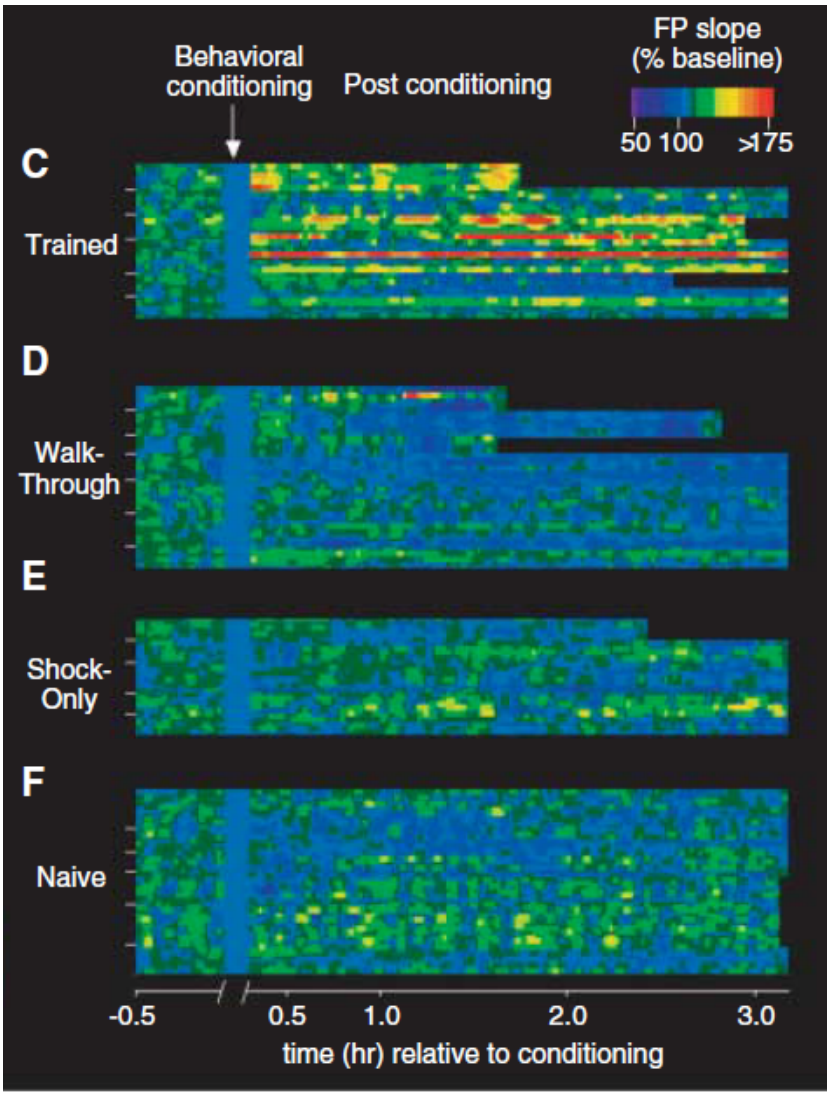

Figure 8. Measurement from CA1 hippocampal cell electrodes in all conditions before and after learning (or control). Individual rats are represented by tick-marks on the left of the graph. Color coding shows a tendency for only those rats in the training condition to show significant

deviations from baseline following learning (yellow and red in *C*). From Whitlock et al. (2006); reprinted with permission from Dr. Mark Bear.

So Whitlock et al. (2006) shows promise, if it can be replicated consistently in future studies, of being a finding that could cement Bliss and Lømo's (1973) original finding into scientific history. Other studies have also suggested learning induced LTP (e.g. in the neocortex: Rioult-Pedotti, Friedman, & Donoghue, 2000; in the amygdala: Rogan, Staubli, & LeDoux, 1997; *in vitro*: McKernan & Shinnick-Gallagher, 1997), but only Whitlock et al. (2006) did so in the hippocampus—so the direct analogy to Bliss and Lømo's original work to my knowledge is yet to be replicated. However, no systematic replications of Whitlock et al. have appeared in the decade that followed. In additional, Whitlock et al measured changes in the population spikes in neurons and LTP is only one of several mechanisms that could explain the increases in the population spike. However, Mitsushima et al. (2011) provided additional evidence for the hypothesis that behavioral learning and memory—specifically inhibitory avoidance learning—involves LTP-like changes at specific synapses within the hippocampus.

Ontogenetic techniques have recently been used by Nabavi et al. (2014) in an attempt to show a link between LTP, and its counterpart LTD, and memory. Despite the new technology, potential confounds exists in such studies, such as: even if stimulating neurons (mimicking LTP) leads to mechanisms that give a plausible explanation to how memory is stored, it does not mean that the reverse causal relationship is also true (that the laying down of a memory involves LTP). Nabavi et al fell short of demonstrating that inducing LTP at an identified set of synapses in an animal was sufficient to "implant" a new memory (in this case, fear of a tone). They found that optically induced LTD at this site efficiently disrupted a previous fear memory, and provided evidence that LTP is necessary for fear learning. A reduction in the level of LTP produced a corresponding decrease in the fear memory indicated that memory is dependent on the level of LTP at these synapses. Nabavi et al showed that LTD was sufficient to inactivate an acquired fear memory, but LTP was not sufficient to implant a fear memory response in a behaviorally naive animal. Also note that Nabavi et al were not directly measuring LTP in the standard sense of a strengthening the synaptic response, but rather associated mechanisms (AMPA to NMDA ratio change) that are related to *in vitro* LTP work in the hippocampus.

The evidence linking any specific set of mechanisms to long term memory is still uncertain. Indeed, the idea that LTP is equivalent to memory has been questioned by Bramham (2010) and several others. The issue of causality in the relationship between LTP and memory is still unresolved. However, one promising proposal, involving LTP-like mechanisms, is that long term memory involves the remodeling of the extracellular matrix that surround neurons in the brain (Tsien, 2013; via "holes" in the perineuronal net).

Given the size of the field of memory, maintaining an informed skepticism of all the branches is a time-consuming challenge that takes years to develop and maintain. For that reason I am skeptical but still wary of how to evaluate some lines of highly domain specific, technical, and occasionally obscurely written research. From my standpoint as an applied memory researcher, not a neuroscientist, my evaluation is that learning has probably been shown to correlate with LTP in some animals, but that the data are messy in such a way that future replication is needed. And even if memory does involve LTP (and LTD), this does not mean that this is the only way that memory is stored (e.g., see Routtenberg, Tabatadze, & McGonigal, 2012, for an alternative, although it should be noted that there seems to be no *widely accepted* alternative theory to LTP and synaptic plasticity).

Summary and Conclusion

To set the Bliss and Lømo (1973) discovery of LTP in context, it was important to outline the precedent theories of synaptic information storage proposed by the likes of Bain, James, Cajal, and Hebb. Without these precedent theories, Bliss and Lømo would have not had a conceptual framework by which to know the importance of their own empirical work. For example, they would not have been able to evaluate why an especially long effect of potentiation was a crucial empirical gap that had strong connection to theory. Bliss and Lømo may not have persisted in their research program had they not known of Cajal's and Hebb's theory. Almost equally important were precedent empirical findings: especially Lloyd's (1949) work with frequency potentiation, and Brenda Milner's identification of the hippocampal area as being important in memory.

After summarizing the main findings of Bliss and Lømo (1973), namely the strengthening of hippocampal synapses for hours, it was then necessary to carefully weigh the evidence for LTP in the decades that followed. This was crucial in order to evaluate the original Bliss and Lømo (1973) discovery. Important to this endeavor was establishing the replicability, generalizability, and mechanisms of LTP (e.g., involving neurotransmitters, receptors, protein transcription, etc.). The research in this respect supported the original findings. Perhaps most importantly was considering the evidence for behavioral learning being causally linked to LTP in the hippocampus in a live animal. For this reason, considerable attention was given to what may the best candidate for such evidence: Whitlock et al. (2006). Whitlock et al. is promising data for a learning-LTP link, though inconsistent and in need of consistent replication. In light of all these and other findings purporting to show a learning-LTP link, I consequently conclude that at the time of writing Bliss and Lømo's (1973) discovery has survived a skeptical examination and is tentatively maintained as a major breakthrough in human scientific history.

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References

Abraham, W. C., Demmer, J., Richardson, C., Williams, J., Lawlor, P., Mason, S. E., Tate, W. P. & Dragunow, M. (1993). Correlations between immediate early gene induction and the persistence of long-term potentiation. *Neuroscience, 56*, 717–727.

Abraham, W. C., Greenwood, J. M., Logan, B. L., Mason-Parker, S. E. & Dragunow, M. (2002). Induction and experience-dependent reversal of stable LTP lasting months in the hippocampus. *Journal of Neuroscience, 22*, 9626–9634.

Andersen, P., Holmqvist, B., & Voorhoeve, P. E. (1966). Entorhinal activation of dentate granule cells. *Acta Physiologica Scandinavica*, *66*, 448–460.

Bain, A. (1872). *Mind and Body: The Theories of Their Relation.* London, UK: Henry S. King.

Baranyi, A., Szente, M. B., & Woody, C. D. (1991). Properties of associative long-lasting potentiation induced by cellular conditioning in the motor cortex of conscious cats. *Neuroscience*, *42*, 321–334.

Bliss, T. V. P. & Lomo, T. (1970). Plasticity in a monosynaptic cortical pathway. *The Journal of Physiology, 207*, 61–61.

Bramham, C. R. (2010). LTP ≠ learning: lessons from short-term plasticity. *Frontiers in behavioral neuroscience*, *4*. Article 3, doi: 10.3389/neuro.08.003.2010

Cajal, S. R. Y. (1894). The Croonian Lecture: La Fine Structure des Centres Nerveux. *Proceedings of the Royal Society of London, 55*, 444–468.

Canal, C. E., Chang, Q., & Gold, P. E. (2007). Amnesia produced by altered release of neurotransmitters after intraamygdala injections of a protein synthesis inhibitor. *Proceedings of the National Academy of Sciences*, *104*, 12500–12505.

Dragunow, M., Abraham, W. C., Goulding, M., Mason, S. E., Robertson, H. A., & Faull, R. L. M. (1989). Long-term potentiation and the induction of c-fos mRNA and proteins in the dentate gyrus of unanesthetized rats. *Neuroscience Letters*, *101*, 274-280.

Eccles, J. C. (1953). *The Neurophysiological Basis of Mind.* Oxford, UK: Oxford University Press.

Esser, S. K., Huber, R., Massimini, M., Peterson, M. J., Ferrarelli, F., & Tononi, G. (2006). A direct demonstration of cortical LTP in humans: a combined TMS/EEG study. *Brain Research Bulletin*, *69*, 86–94.

Rogers, J. (2017). Harness your Hebbian Power: Scientists find real-life superpower hidden deep in our brains. *Express* [Newspaper]. Retrieved from http://www.express.co.uk/news/science/774840/Super-Human-gene-networks-people-learn-evolve-hebbian

Feldman, D. E. (2000). Timing-based LTP and LTD at vertical inputs to layer II/III pyramidal cells in rat barrel cortex. *Neuron*, *27*, 45–56.

Feldman, D. E., Nicoll, R. A., & Malenka, R. C. (1999). Synaptic plasticity at thalamocortical synapses in developing rat somatosensory cortex: LTP, LTD, and silent synapses. *Journal of Neurobiology*, *41*, 92–101.

Gloor, P., Vera, C. L., & Sperti, L. (1964). Electrophysiological studies of hippocampal neurons. III. Responses of hippocampal neurons to repetitive perforant path volleys. *Electroencephalography & Clinical Neurophysiology, 17*, 353–370.

Green, J. D. & Adey, W. R. (1956) Electrophysiological studies of hippocampal connections and excitability. *Electroencephalography & Clinical Neurophysiology, 8*, 245–262.

Hebb, D. O. (1949). *Organization of Behavior: a Neuropsychological Theory*. New York: John Wiley.

Jeffery, K. J., Abraham, W. C., Dragunow, M. & Mason, S. E. (1990). Induction of fos-like immunoreactivity and the maintenance of long-term potentiation in the dentate gyrus of unanesthetized rats. *Molecular Brain Research, 8*, 267–274.

Jeffery, K. J., Abraham, W. C., Dragunow, M., & Mason, S. E. (1990). Induction of Fos-like immunoreactivity and the maintenance of long-term potentiation in the dentate gyrus of unanesthetized rats. *Molecular Brain Research*, *8*, 267–274.

Kandel, E. R., & Tauc, L. (1964). Mechanism of prolonged heterosynaptic facilitation. *Nature*, *202*, 145–147.

Kirkwood, A., Lee, H. K., & Bear, M. F. (1995). Co-regulation of long-term potentiation and experience-dependent synaptic plasticity in visual cortex by age and experience. *Nature*, *375*, 328–331.

Krug, M., Lossner, B. & Ott, T. (1984). Anisomycin blocks the late phase of long-term potentiation in the dentate gyrus of freely moving rats. *Brain Research Bulletin, 13*, 39–42.

Lashley, K. S. (1950). In search of the engram. *Society of Experimental Biology Symposium No.4: Physiological Mechanisms in Animal Behaviour.* Cambridge, UK: Cambridge University Press.

Liu, X. G., & Sandkühler, J. (1995). Long-term potentiation of C-fiber-evoked potentials in the rat spinal dorsal horn is prevented by spinal N-methyl-D-aspartic acid receptor blockage. *Neuroscience Letters*, *191*, 43–46.

Lloyd, D. P. C. (1949). Post-tetanic potentiation of response to in monosynaptic reflex pathways of the spinal cord. *Journal of General Physiology, 33*, 147–170.

Lømo, T. (1966). Frequency potentiation of excitatory synaptic activity in the dentate area of the hippocampal formation. *Acta Physiol. Scand., 68* (Suppl. 277), 128.

Lømo, T. (1971a). Patterns of activation in a monosynaptic cortical pathway: the perforant path input to the dentate area of the hippocampal formation. *Experimental Brain Research, 12*, 18–45.

Lømo, T. (1971b). Potentiation of monosynaptic EPSPs in the perforant path–dentate granule cell synapse. *Experimental Brain Research, 12*, 46–63.

Lømo, T. (2003). The discovery of long-term potentiation. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences*, *358*, 617–620.

Lucas, G. (1977). *Star Wars.* [Motion picture]. Lucasfilm Ltd. San Francisco, CA.

McKernan, M. G. & Shinnick-Gallagher, P. (1997). Fear conditioning induces a lasting potentiation of synaptic currents *in vitro. Nature, 390*, 607–611.

Mitsushima, D., Ishihara, K., Sano, A., Kessels, H. W., & Takahashi, T. (2011). Contextual learning requires synaptic AMPA receptor delivery in the hippocampus. *Proceedings of the National Academy of Sciences*, *108*, 12503–12508.

Nabavi, S., Fox, R., Proulx, C. D., Lin, J. Y., Tsien, R. Y., & Malinow, R. (2014). Engineering a memory with LTD and LTP. *Nature*, *511*, 348–352.

Nguyen, P. V., Abel, T. & Kandel, E. R. (1994). Requirement of a critical period of transcription for induction of a late phase of LTP. *Science, 265*, 1104–1107.

Nicoll, R. A., & Roche, K. W. (2013). Long-term potentiation: peeling the onion. *Neuropharmacology*, *74*, 18–22.

Otani, S., Marshall, C. J., Tate, W., Goddard, G. V. & Abraham, W. C. (1989). Maintenance of long-term potentiation in rat dentate gyrus requires protein synthesis but not mRNA synthesis immediately post-tetanization. *Neuroscience, 28*, 519–526.

Racine, R. J., Milgram, N. W. & Hafner, S. (1983). Long-term potentiation phenomena in the rat limbic forebrain. *Brain Research, 260*, 217–231.

Rioult-Pedotti, M. S., Friedman, D., & Donoghue, J. P. (2000). Learning-induced LTP in neocortex. *Science*, *290*, 533–536.

Robinson, G. B., & Reed, G. D. (1992). Effect of MK-801 on the induction and subsequent decay of long-term potentiation in the unanesthetized rabbit hippocampal dentate gyrus. *Brain research*, *569*, 78–85.

Rogan, M. T., Staubli, U. V. & LeDoux, J. E. (1997). Fear conditioning induces associative long-term potentiation in the amygdala. *Nature, 390*, 604–607

Routtenberg, A., & Rekart, J. L. (2005). Post-translational protein modification as the substrate for long-lasting memory. *Trends in Neurosciences*, *28*, 12–19.

Routtenberg, A., Tabatadze, N., & McGonigal, R. (2012). Delayed yet persistent hippocampal molecular and structural alteration after learning: New players in the debate on time-dependent long-lasting memory processes. *Hippocampus*, *22*, 1225–1227.

Scoville, W. B., & Milner, B. (1957). Loss of recent memory after bilateral hippocampal lesions. *Journal of Neurology, Neurosurgery, and Psychiatry*, *20*, 11–21.

Sharma, A. V., Nargang, F. E., & Dickson, C. T. (2012). Neurosilence: profound suppression of neural activity following intracerebral administration of the protein synthesis inhibitor anisomycin. *Journal of Neuroscience*, *32*, 2377–2387.

Staubli, U. & Lynch, G. (1987). Stable hippocampal long-term potentiation elicited by 'theta' pattern stimulation. *Brain Research, 435*, 227–234.

Tsien, R. Y. (2013). Very long-term memories may be stored in the pattern of holes in the perineuronal net. *Proceedings of the National Academy of Sciences*, *110*, 12456–12461.

Whitlock, J. R., Heynen, A. J., Shuler, M. G., & Bear, M. F. (2006). Learning induces long-term potentiation in the hippocampus. *Science*, *313*, 1093–1097.